LABORATORY PRACTICE

RESEARCH · CONTROL · TEACHING
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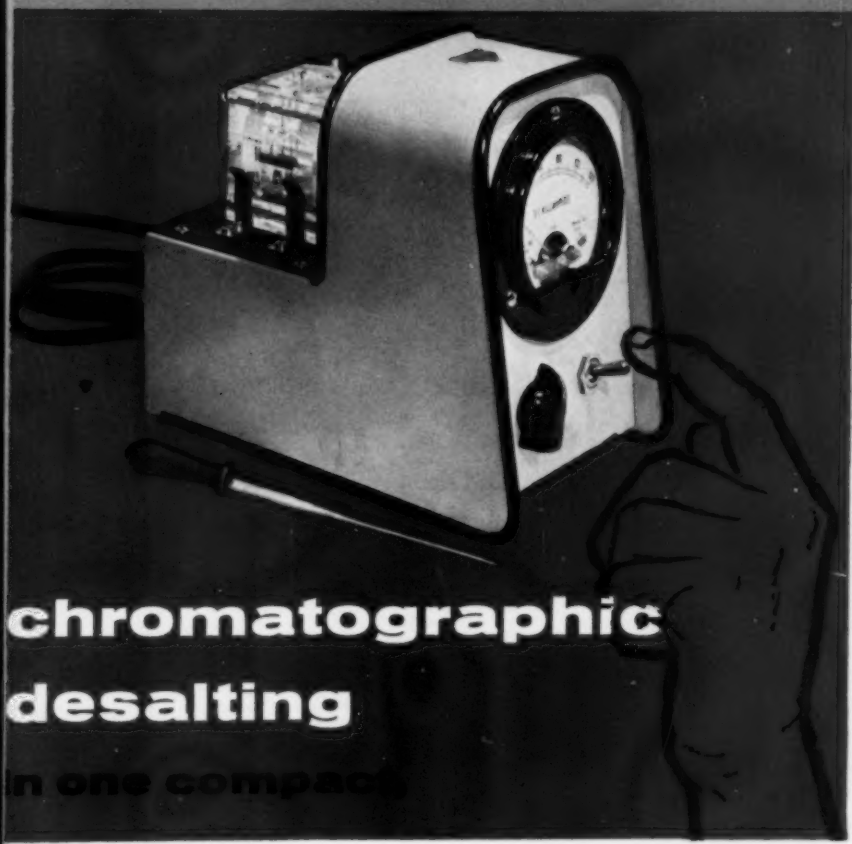

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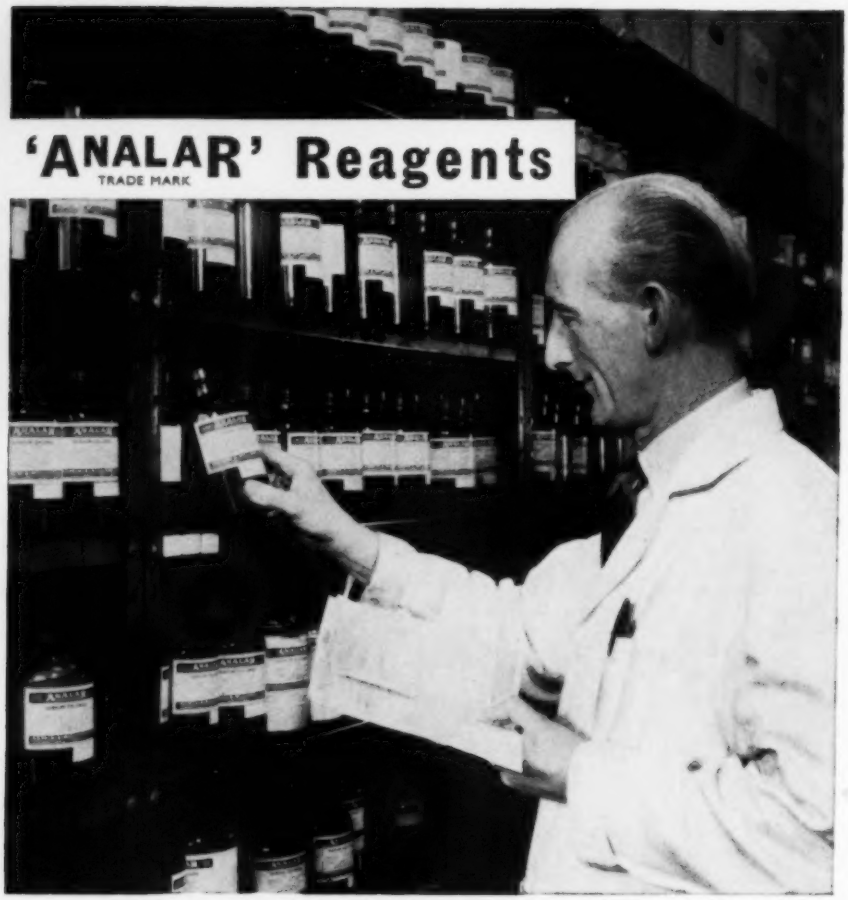

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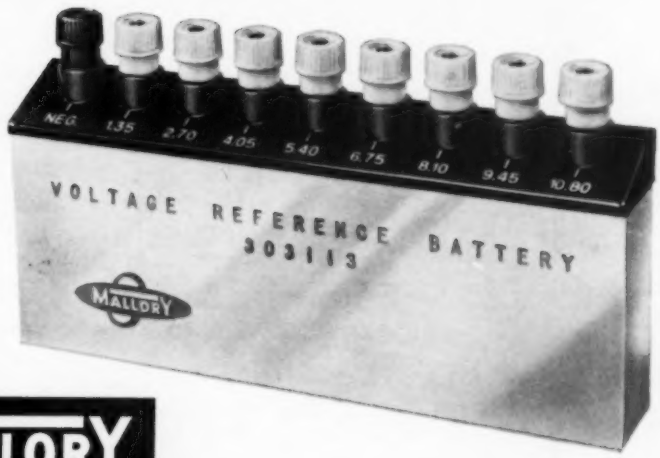

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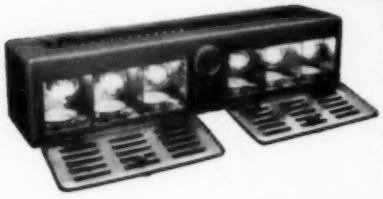

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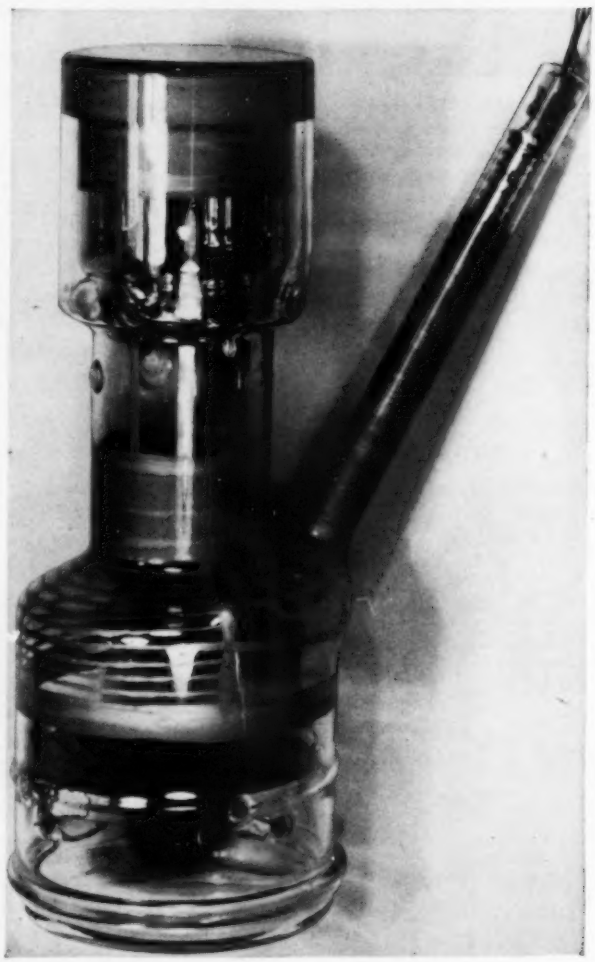

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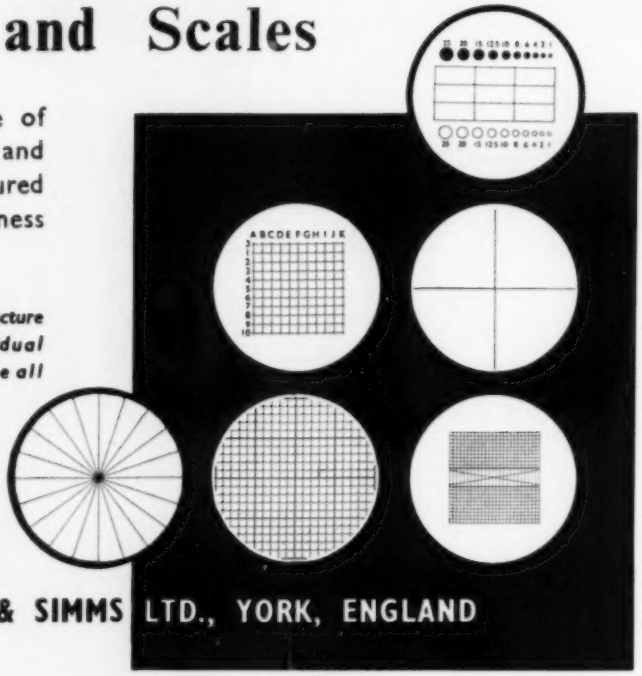

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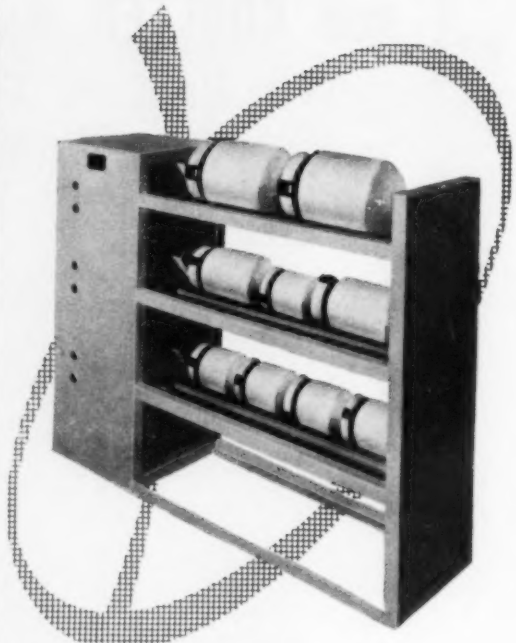

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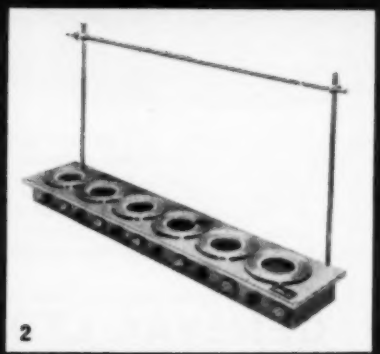
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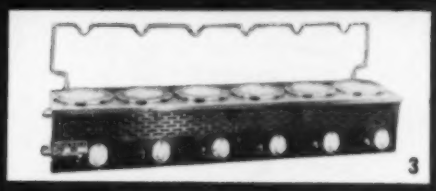
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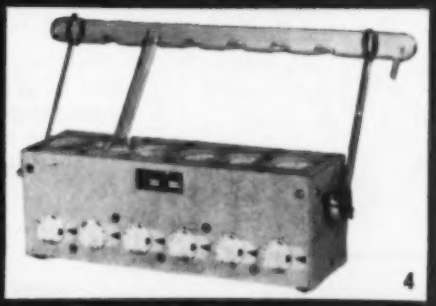
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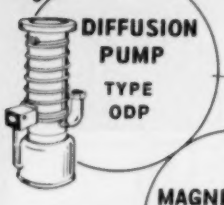
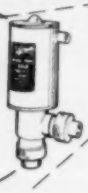
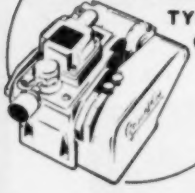


DIFFUSION PUMP TYPE ODP



MAGNETIC VALVE TYPE SV



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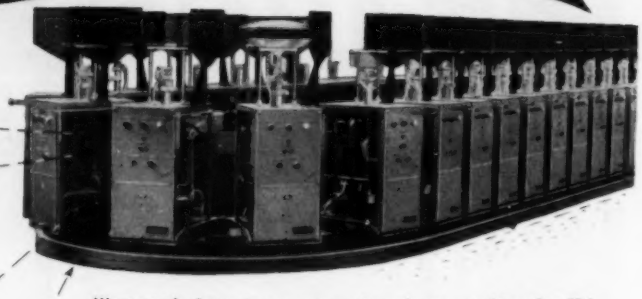

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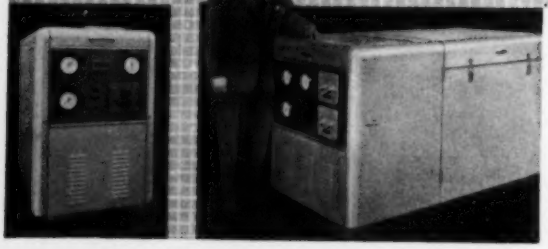

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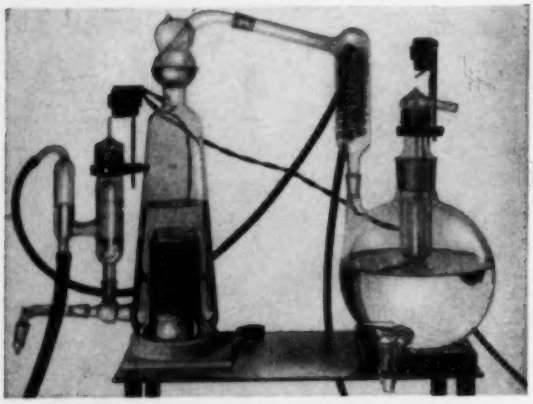

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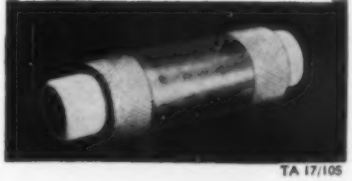

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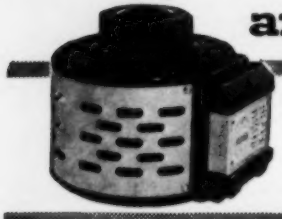

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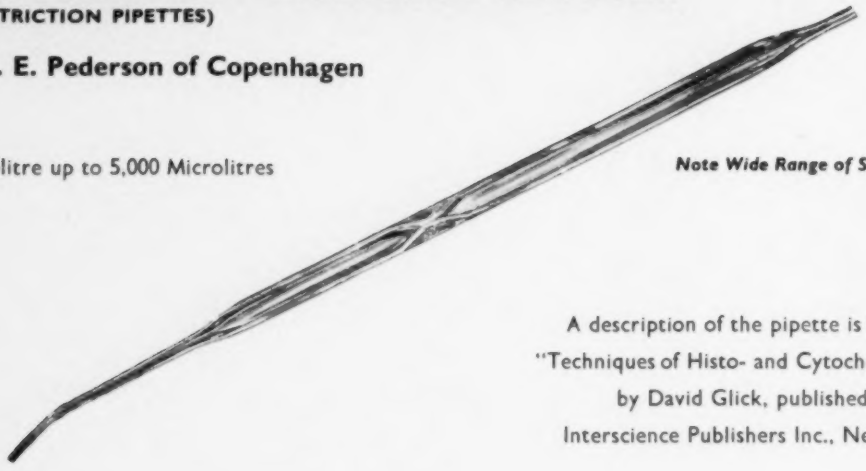

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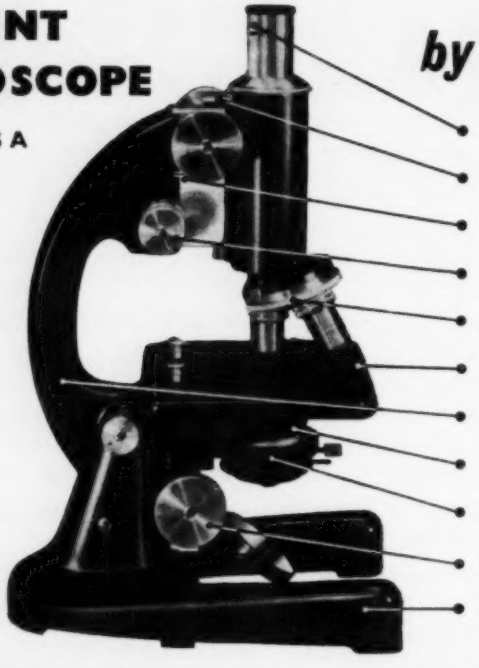

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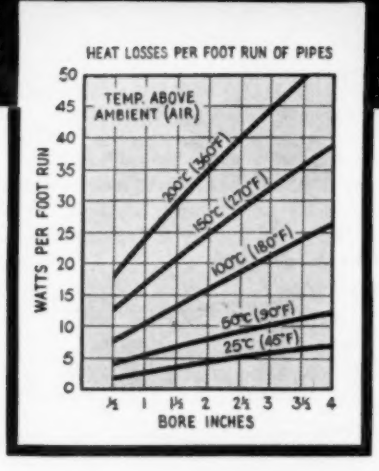

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EDITORIAL

The Fifth International Biochemistry Congress

THIS was held in Moscow in August. A full scientific report will appear in our November issue but in view of the interest attaching to a congress held in U.S.S.R. we take the opportunity to record our impressions of the congress generally and of Leningrad and Moscow in particular. It is obviously absurd to attempt to record impressions of a vast country on the basis of a few days spent in its two largest cities.

Of the organization of the Congress itself we have nothing but the highest praise. It was held in the new University building on Lenin Hills and most efficiently run. Directions for reaching the various lecture theatres were liberally displayed, and for a congress involving some 5,000 participants this alone was no mean feat. The University was truly a majestic building nearly 800 feet high and with other dimensions in keeping.

The departments of Chemistry, Physics, and Biology and Soil Science were huge buildings in themselves; one got quite a lot of exercise in going from one theatre to another. The shuttle bus service from the hotels to the University was most efficient and every effort was made to help members of the Congress in getting about, few of whom spoke Russian. Abstracts of nearly 6,000 communications were available in one volume issued beforehand to all members, a most useful assistance. Unfortunately many of these were in Russian, and so were useless to most of us.

Moscow has many new buildings, mostly blocks of flats. There are a number of gigantic buildings—public offices, hotels and universities—favouring what we should call the 'wedding cake' type of architecture. Marble is used considerably for the interior, which gives an atmosphere of grandeur.

The new Metro is likewise plentifully decorated in marble for the halls and corridors. The electric trains themselves are as modern and efficient as any we have seen, and the photocell controlled turn-styles for passengers strike a new note in city transport. The Moscow-Volga canal is also a magnificent achievement, and in engineering and public works the Russians are clearly unsurpassed.

The visitor will appreciate the fine rooms in the good hotels, and the services of the Intourist guides, who are mostly young women graduates who speak very good English. The conducted tours are well run.

However, not all impressions of Russian life were as favourable as those so far mentioned. It is perhaps not fair to criticize the food because this is obviously a matter of national taste. We are accustomed to more variety in all courses, and we like hot dishes to be really hot. The worst aspect of dining in U.S.S.R. is unquestionably the service which is unbelievably bad. It may take anything up to two hours to get a simple type of meal. There seems to be no head waiter or manager available to receive complaints, but as most visitors cannot speak Russian it would be difficult to complain. The waiters are completely indifferent to the diners; perhaps the absence of tipping is a factor to be considered.

All arrangements for visitors are in the hands of Intourist, and are highly centralized. The guides and receptionists are very pleasant and helpful, but it is difficult to find out what arrangements have been made. One just arrives and waits until at last some definite information is forthcoming. The guides cannot do anything without information and instructions. Efforts to find out are only rewarded at the latest possible moment; 'Come back later' or 'Ask again tomorrow' is the answer that all tourists get as they queue to find out where and when they are to go. Coupled with this system is their practice of taking all documents and passports from the tourist on arrival, and only issuing the new documents at the latest possible moment. It is not surprising that those accustomed to having all travel details firmly fixed in advance develop a slight anxiety neurosis before the trip ends.

Unless one has previously established personal contact with Russian scientists in one's own field, it is hopeless to try and meet them for informal discussion. Everything has to be done through Intourist, and the process is so slow that one has left the country before hearing anything. The post is likewise hopelessly slow. It is incidentally notorious that letters to scientists in Iron Curtain countries are only rarely answered.

From the little contact we had with Soviet citizens we formed the impression that fundamentally they are most willing to be friendly and helpful; it is the machinery which makes intercourse difficult. A much freer exchange of ideas and visits between our two countries is greatly to be desired. More visitors would be attracted to Russia if the hotel service were improved, and the general communication system speeded up. British scientists should be able to visit their opposite numbers in Moscow and elsewhere with much greater ease, and postal communication should be speedy and unrestricted. Both countries have much to learn from each other.

We have already expressed the opinion that international congresses are now so unwieldy that entirely new thinking is needed. Small informal symposia for which a few scientists from a number of countries could congregate in various countries in turn, would in our view afford those opportunities for friendly discussion for which the present gigantic congress is quite unsuited. The proceedings of this Congress are to be published at a cost of about £70. Even if this scientific information were not published elsewhere, this seems a high price for papers given at one congress.

THE BIOLOGICAL ASSAY OF INSECTICIDES

by James R. Busvine, D.Sc., M.I.Biol.

London School of Hygiene and Tropical Medicine

General Principles

The biological assay of insecticides covers a wide field, ranging from ingenious ways of treating individual insects in the laboratory to field trials which may cover large areas of country. A great variety of test methods is involved, partly owing to the big differences in scale just mentioned, but also because of the different requirements of insecticides acting as stomach poisons, contact poisons or fumigants. In addition to this necessarily wide range of test methods, the subject has been complicated in the past by the rather anarchic disposition of some investigators who seemed to find it discreditable to use a test method invented by anyone else. Consequently, much research time was spent devising new apparatus, without any specially new principles, and the literature abounds in trivial variations of some techniques. However, it is now beginning to be appreciated that there are considerable advantages in the general adoption of standard test methods. These allow the results of one worker to be compared directly with those of another. The importance of this grows with the increased use of insecticides and the emergence of international problems, such as aircraft disinsection and insecticide resistance. Insecticide testing has been somewhat systematized in recent years by the appearance of text-books on the subject. There is a British monograph (Busvine, 1957) and a symposium published in the U.S.A., of which two volumes have appeared (Shepard, 1958, 1960).

In devising or in choosing a suitable test method, the object of the experiment must be borne in mind. Biological testing of insecticides is usually done for one of the following reasons:

(i) *The discovery and development of new insecticides.*—This type of investigation has been proceeding on a considerable scale for some 25 years in the laboratories of several large chemical firms as well as government research stations in various parts of the world. It must be admitted that much of the work is empirical so that the first stage consists in screening large numbers of miscellaneous organic compounds. In the first place simple, rather crude tests can be used, which consist of exposing different types of insects to high concentrations of the samples. Diverse insect types are used to avoid possible bias due to specific susceptibility. The species commonly used are those easy to rear in the laboratory, e.g. stored product beetles (grain weevil, flour beetle), housefly, bed bug, yellow fever mosquito.

The more promising samples selected in this way are subjected to more careful evaluation and then to small scale trials under conditions simulating, to some extent, practical usage. The final testing requires large field trials and the whole process of discovering and completely developing a new insecticide is surprisingly expensive.

James R. Busvine, D.Sc., M.I.Biol.

(ii) *Measurement of insecticide residues by bioassay.*—In experiments of this type, the insects are used as a tool to measure minute quantities of insecticide. The object may be to assess the actual insecticidal powers of residues in the field (e.g. the World Health Organization (W.H.O.) test for mosquito-killing powers of walls in sprayed dwellings). Alternatively the insects may be used instead of chemical estimation, to detect residues on treated food (e.g. fruit) which may be harmful to man. The advantage of using insects for this purpose is that they are practically as sensitive as chemical tests and they usually react only to the toxic material. Their disadvantage is their greater variability compared with chemical reagents.

The present status of residue estimation by bioassay was helpfully reviewed by Needham (1960) who visited a number of laboratories in Europe and reported on

the methods in use. These generally belong to one of the following types:

(*a*) direct methods, in which the test organisms are confined over a pulp of the residue-containing material; (*b*) film methods, in which the test organism is exposed to the residue after evaporation of a solvent which has been used to extract it from the sample; and (*c*) aqueous methods, in which the residue is extracted from the sample and then added to water containing an aquatic test organism.

As regards the test organism, an important requisite is that it can be reared conveniently and rapidly in the laboratory. For test methods (*a*) and (*b*) just mentioned, the fruit fly, *Drosophila melanogaster*, is a common choice; ordinary houseflies can also be used. For method (*c*) the larvae of the mosquito *Aedes aegypti* are convenient; alternatives are the water flea, *Daphnia spp.*, or certain fish, such as guppies (*Lebistes reticulatus*).

Bioassay is generally regarded as a rapid and convenient method but it does not satisfactorily replace chemical analysis of residues in all respects.

(iii) *Detection and Measurement of Insecticide Resistance.*—The widespread emergence of strains of insects resistant to modern insecticides has called for standard testing methods to detect and confirm their presence. Such tests are normally designed for use in the field, but require a fair standard of precision to be useful. A number of standard tests for resistance in pests of medical importance have been adopted for international use by the Expert Committee on Insecticides of the World Health Organization (W.H.O. 1960). Tests were first devised for the more important disease vectors, e.g. human lice, adult and larval mosquitoes. These have been widely used during the past few years. Other tests are in process of adoption for fleas, bed bugs, houseflies, sandflies and certain other insects.

These three objectives differ in their requirements of the balance to be struck between (*a*) strictly standardized (and therefore probably artificial) conditions, allowing exact and repeatable tests, and (*b*) conditions which simulate the field environment, permitting some prediction of the practical performance of an insecticide. Type (*a*) conditions are suitable for bioassay and resistance tests; whereas type (*b*) must be given priority at some stage in the development of new insecticides.

The standardization of bioassay tests involves not only the usual attention to chemical and physical aspects of the experiment, but also the use of insects in a consistent and reliable state. This is not easy, because insects are very plastic physiologically and are very sensitive to changes in temperature and nutrition. They are, indeed, easy to rear and some workers attempt to make up for their variability by the use of large numbers; but this is not a very satisfactory solution.

The difficulty in ensuring exact standardization of the insect material is unfortunate, not only on its own account but also because it sometimes induces a generally uncritical outlook. As a certain amount of 'biological variability' is expected the experimenter feels absolved from attempting physical precision. It is especially unfortunate if this attitude prevents an intelligent study of all the mechanical, physical and chemical aspects of the test. This point will be illustrated in the next section.

Description of some Testing Methods

This article deals only with contact insecticides, because they seem to be increasingly important; and with insects of public health importance, as the writer is most familiar with these.

Laboratory tests.—The potency of an insecticide can be measured by the doses required to kill various insects or the time required to kill them after a standard dose. For various reasons it is usually more convenient to make comparisons on the basis of dosage rather than speed of action. Accordingly the common practice is to apply various doses to batches of insects and inspect them at some later period to determine what proportion has been killed. Curiously enough this is not always easy, because death in insects is often less clear cut than in higher animals. Some robust insects (beetles, cockroaches) can remain paralysed for long periods without being evidently dead or alive. Accordingly it may be necessary to specify a particular level of intoxication (which should normally terminate in death) and use this to categorize the treated batch.

The next problem is that of dosage. Because insects are so small it is by no means easy to give them measured doses individually, as would be done with a larger experimental animal. Consequently one method of standard treatment is to make the insects 'dose themselves' by putting them into environments treated with various concentrations of poison, for limited periods. We shall consider some examples of this method and then proceed to more precise experimental methods in which attempts are made to dose individuals or groups of insects.

Methods in which the Insects dose Themselves

Surfaces.—Crawling insects can be confined on surfaces treated in various ways. In the W.H.O. test for resistance in body lice, the insects are confined for 24 hours on cloth which has been treated with powder insecticides at different rates. Several test methods involve confining insects of various types on residues of insecticides left after evaporation of acetone solutions. Such residues may be deposited on glass, filter paper, etc. Later it was recognized that such dry crystalline deposits were very variable and difficult to duplicate. More reliable results have been obtained by dissolving the insecticides in a non-volatile oil and depositing a fine film of these oil solutions on filter paper or glass (Busvine/Nash test and W.H.O. test for resistance in mosquitoes, Fig. 1).

Volumes.—Flying insects may be attacked by insecticides in the form of aerosols. A well-known test method for household insecticides is the Peet-Grady method, in which houseflies are confined in a 6 ft. cubic chamber

Fig. 1. World Health Organization method of measuring resistance in adult mosquitoes.

into which aerosol is sprayed (Fig. 2). This test was originally designed to compare the efficacy of household insecticides. Apart from the final killing action, the speed of 'knock down' is also important; and this can be measured by the numbers paralysed at the end of 10 minutes.

Aquatic insects can be dosed by immersing them in suspensions of insecticide at different rates for a standard time; for example, the W.H.O. standard test method for resistance in mosquito larvae.

General comments.—It will be realized that in none of the methods mentioned do we know the actual dose of insecticide, which contaminates the individual insects. If one speaks of the rate of deposit or concentration of aerosol or suspension as a 'dose', one must never forget the inverted commas, which indicate that the word is only an unknown function of the true dose. Also it should be remembered that the dose acquired may be affected by environmental and other conditions quite independently of the actual poisoning process. For example, an increase in temperature generally augments the 'pick-up' of insecticide in various tests, owing to increased activity, etc. But while a rise in temperature accentuates poisoning with some poisons, such as BHC or dieldrin, it decreases the effects of DDT. Therefore in tests where insects 'dose themselves' there is a strong temperature coefficient with most insecticides, but with DDT temperature affects pick-up and poisoning in different directions, so that the overall effect is negligible.

Pick-up of insecticide is also important in aerosol tests. As pyrethrum stimulates the insects to fly, the pick-up of droplets is likely to be much bigger when pyrethrins are present in a mixed aerosol.

Methods Attempting to give Known Doses

As far as contact insecticides are concerned the first method which attempted to give known doses to insects was the spray tower. A variety of different spraying towers has been designed (Fig. 3) which permit either spraying directly at the insects or indirectly into a closed space to provide a mist which is then allowed to settle down on to the insects. In both methods the object is to cover the insects with a fine deposit, which is even over the treated surface, so that each gets the same dose. This can be calculated from the surface area.

More precise than the spray tower is the topical application syringe, of which there are several different types. One variety uses a small glass syringe operated by a micrometer screw. Another form uses capillary tubing drawn to a point, the dose applied being measured by movement of the meniscus along the tubing. The dose can be driven out by air pressure or

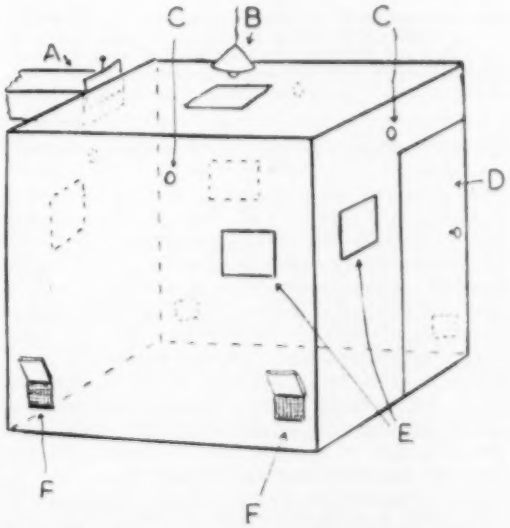


Fig. 2. The Peet-Grady chamber for testing aerosols against houseflies. A, extractor duct; B, light; C, holes for introducing spray; D, door; E, observation windows; F, vents.

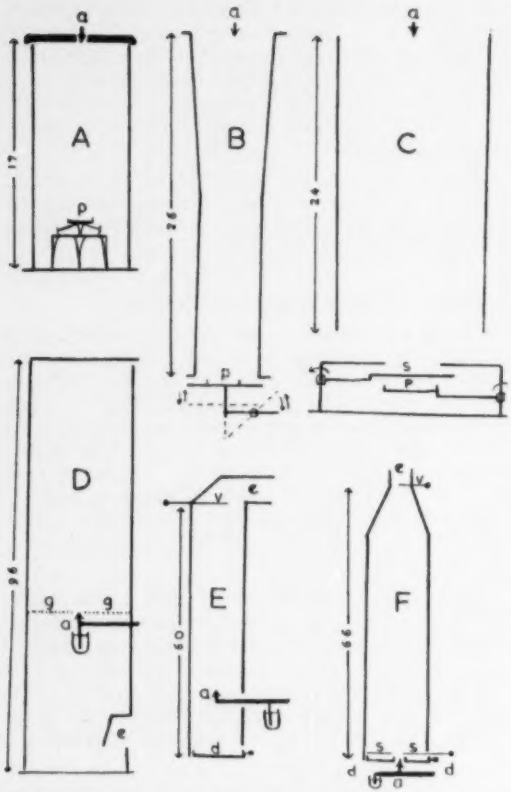


Fig. 3. Spraying towers. A, Tattersfield and Morris; B, Potter; C, ten Houten and Kraak; D, McGovran and Mayer; E, McCallan and Wilcoxon; F, Way. Heights given in inches. a, spray gun; p, petri dish target; s, movable shield; g, wire mesh target; e, exit duct for mist; v, valve; d, drawer target.

the liquid simply allowed to flow out over the insect on contact.

A somewhat more elaborate method of dosing developed by P. S. Hewlett is a drop-shooting apparatus. Very tiny quantities of liquid expelled from a fine hypodermic syringe needle are shot off by a controlled jet of air, on to an insect placed on the target. (Fig. 4).

General Comments.—The method of individual dosing gives more repeatable results than tests in which insects dose themselves. This fact has led to its increasing use for basic laboratory studies on insects as big as a housefly or larger. Although it is possible to use the technique with smaller insects (e.g. mosquitoes) the difficulties of applying the very small quantities necessary becomes a serious limitation.

Two other matters require comment. Some form of anaesthesia is required (usually) to facilitate handling of the insects during treatment. Carbon dioxide is commonly used. Finally it is worth remembering that the dose applied to the exterior of the insect is not the ultimate toxic agent. Rate of penetration is an important factor and it has been shown that this varies according to the solvent used to carry the insecticide. Further, it cannot be assumed that penetration is directly proportional to the quantity applied except over a limited range.

Field Trials

The principles governing the planning, conducting and interpreting of field trials are rather beyond the scope of this article, but a few general remarks may be of interest.

Planning.—It is readily apparent that large field trials are very expensive. Less obvious is the fact that, despite the simplicity of the mechanical operations involved, they are usually more difficult to complete successfully than laboratory investigations. Careful planning to ensure adequate replication of treated and control areas is essential, otherwise a lot of hard and expensive work will end in inconclusive results.

Interpretation.—Very often the results of field trials will depend on assessments of insect populations before and after treatment or in treated and untreated areas. This is a difficult technical problem involving a considerable knowledge of the biology of the insect concerned. Control programmes often bring to light the rudimentary nature of our quantitative knowledge of common insect pests.

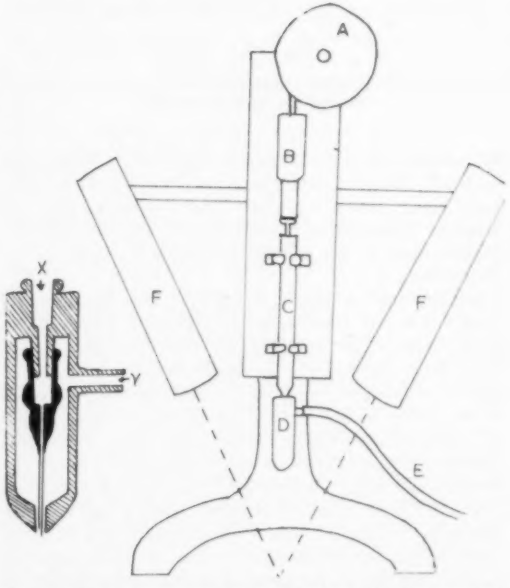


Fig. 4. Micro-drop shooter (after Hewlett). A, disc attached to reduction gear; B, micrometer handle; C, glass syringe; D, 'jet needle'; E, tube supplying air surge; F, lights focusing pointers on target; *Left:* Jet needle section: X, liquid entry; Y, air surge entry.

Statistical Aspects of Laboratory Testing of Insecticides

The quantitative aspect of toxicological assay can involve quite elaborate statistical treatment and there is at least one good text-book on the subject (Finney, 1952). All that will be attempted here is to indicate cardinal relationship between dosage and mortality in the quantal response method, which is normally employed. The basic assumptions are that for each individual insect at any given moment there is a certain 'lethal dose' of poison which will just kill it; with lower doses it will survive and higher doses will be fatal. The second assumption is that, in a homogeneous population of insects, these lethal doses will show a statistically normal distribution because they are dependent on random variability. Finally the dosage must be expressed on a logarithmic scale in conformity to the Weber-Fechner law of the response of biological material to stimuli. This is easier to accept if we consider that raising a dose from one unit to two cannot be comparable with raising it from one hundred and one to one hundred and two units, as when an arithmetic scale is used.

Given these data we can determine the susceptibility levels of a population of insects to a given insecticide by treating batches of them at a series of dosage levels and observing the proportions killed (quantal response). Fig. 5 will show that regular increments of dose (even

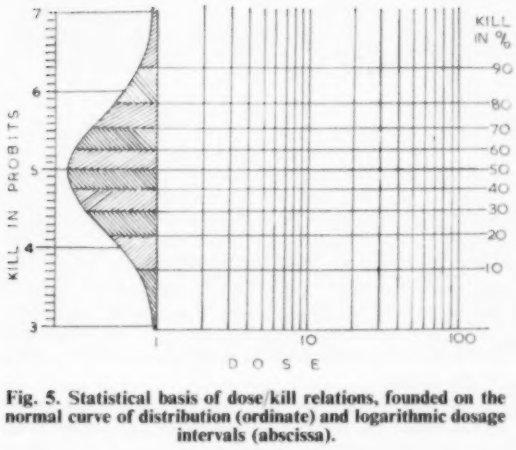


Fig. 5. Statistical basis of dose/kill relations, founded on the normal curve of distribution (ordinate) and logarithmic dosage intervals (abscissa).

on a log. scale) will not kill the same proportions of the population, owing to the grouping of the majority round the mean. The figure shows how the ordinate scale must be distorted to divide the population into equal (10 per cent) proportions, as represented by the areas under the normal curve. Alternatively the percentages can be converted to units based on the normal curve, so that 1 ('probit') unit equals the standard deviation. The mean is equated with 5 for convenience to avoid negative values. With both methods the

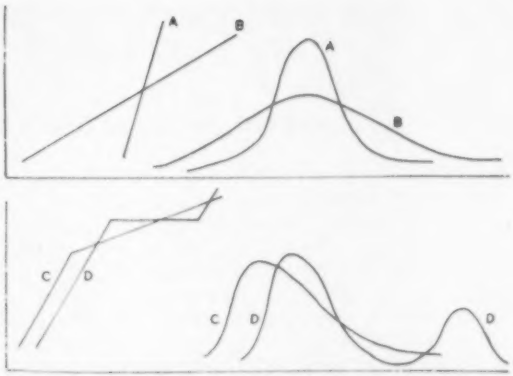


Fig. 6. Interpretation of modified dose/kill curves (on logarithmic probability paper, as in Fig. 5) in terms of distribution curves. *Left:* **the regression lines;** *right:* **the corresponding curves;** *above:* **homogeneous populations;** *below:* **examples of mixed populations.**

results of the tests with batches of insects can be plotted against (log.) dosage to give a straight line. The 50 per cent mortality value, representing mean of the population, is the point of greatest reliability and is normally used for basic toxicological studies. Statistical methods are available for calculating the exact position of a dose/kill regression line, its standard deviation and fiducial limits, but for many purposes a simple graphical solution is adequate.

Fig. 6 illustrates some of the implications of particular types of dosage/mortality curve. Straight lines correspond to normal populations. The steeper they are (the smaller the standard deviation) the less variation is displayed by the population.

Curved or bent lines are produced by mixed populations such as may occur with incipient insecticide resistance. The lower figures show how asymetrical or bimodal population distributions will be reflected in dosage/mortality regression lines.

BIBLIOGRAPHY

Busvine, J. R. (1957). 'Techniques for Testing Insecticides'. 208 pp. Commonwealth Bureau of Entomology, London. £1 10s.

Finney, D. J. (1952). 'Statistical Methods in Bioassay'. 661 pp. Griffin & Co., London.

Needham, P. H. (1960). 'An Investigation into the Use of Bioassay for Pesticide Residues in Foodstuffs'. Analyst, **85**, 792-809.

Shepard, H. H. (Ed.) (1958). 'Methods of Testing Chemicals on Insects'. Vol. I, 356 pp. £2 (1960) *ibid.* Vol. II, 248 pp. Burgess Publ. Co., Minneapolis.

World Health Organization (1960). 'Insecticide Resistance and Vector Control', Wld. Hlth. Org. Techn. Rpt. Ser. No. 191, 98 pp., Geneva. (H.M.S.O. 5s. 1d.).

RHEOLOGICAL TECHNIQUES

The November issue of *Laboratory Practice* will contain the papers read at the meeting of the British Society of Rheology.

THE ELECTRON MICROSCOPE IN STUDIES ON PROTEIN SYNTHESIS

by P. N. Campbell, D.Sc.

Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London, W.1

WHILE my predominant interest is biochemistry and not electron microscopy I believe that collaboration between biochemists and electron microscopists can be very fruitful. The field of protein biosynthesis* is a very good example of the way in which these two groups of workers can work together to increase our understanding of the way living cells synthesize these complex molecules. First, however, it is necessary to consider briefly the salient features of protein structure.

P. S. Campbell, D.Sc.

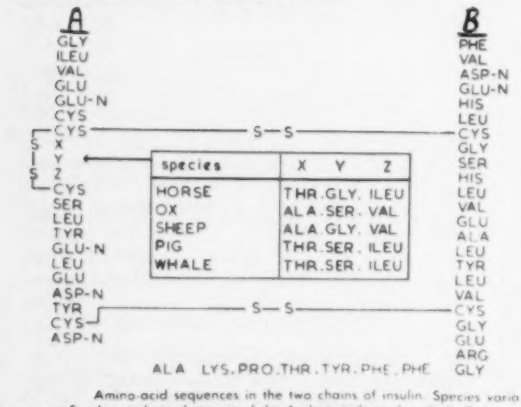
Protein Structure

Proteins contain a wide variety of organic molecules—amino acids, carbohydrates, nucleic acids, lipids, etc.—but so far as protein synthesis is concerned attention has been directed mainly to the amino acids. The amino acids are linked together by peptide bonds to form the polypeptide chains which are the backbone of the protein molecules. These chains may be arranged in three dimensions in different ways giving the molecules a very wide range of properties. The molecular weights of the proteins vary from 6,000 for insulin up to many hundreds of thousand (e.g. thyroglobulin, 600,000). Their physical properties may be similar to those of the soluble globular plasma proteins or they may be insoluble and fibrous, as are the hair or feather proteins.

The amino acids of the polypeptide chains are of 20 different kinds. For the purposes of illustration the amino acid sequence of the polypeptide chains of insulin may be considered (Fig. 1). This sequence was the first to be determined for a protein and was the work of F. Sanger of Cambridge University[2]. Insulin contains two polypeptide chains which are linked through S-S bridges, a common way of linking chains in proteins. The different amino acids are denoted in Fig. 1 by the now usual abbreviations, which consist of the first three letters of the full name (gly = glycine, etc.). The sequence in which the amino acids occur is quite specific for a given protein. Thus all the molecules of horse insulin have exactly the same sequence but differ in the position of one amino acid from those of whale insulin.

Recently much effort has been devoted to the determination of the amino acid sequence of proteins. Thus the complete sequence of ribonuclease is now known and good progress has been made with haemoglobin. In the latter case Ingram has shown[3] that the haemoglobin present in the red blood cells of South African negroes with sickle-cell anaemia, differs from normal haemoglobin by a difference of only one amino acid in a chain of nearly 300 amino acid residues. These findings emphasize the precise structure of proteins.

*The reader is referred to a recent review in which some aspects of the work described are dealt with in greater detail[1].



Amino-acid sequences in the two chains of insulin. Species variation is confined entirely to that part of the A chain indicated as X, Y, Z

Fig. 1.

Relationship between Cell Type and Protein Synthesis

The proteins synthesized by the cells forming the different tissues of the body are characteristic of the

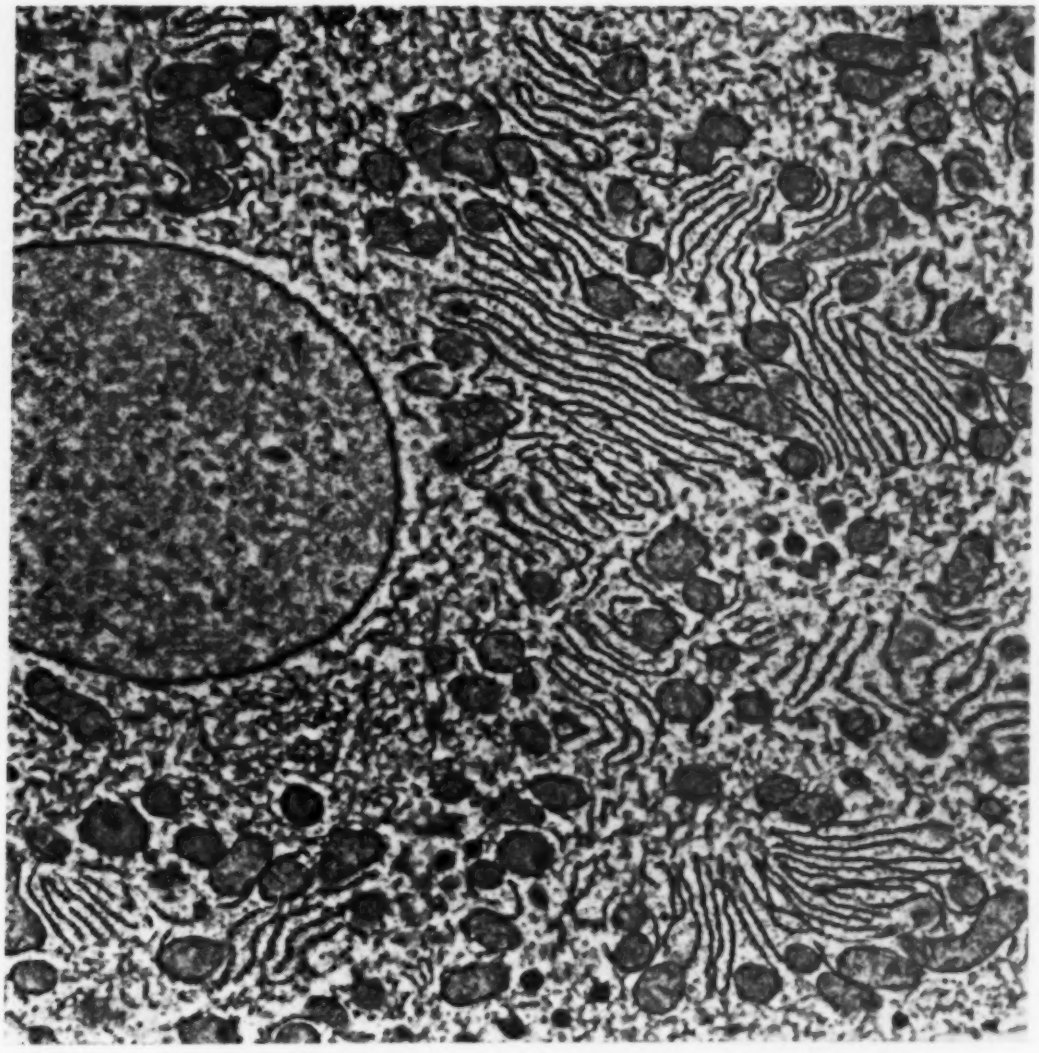


Fig. 2. Survey picture of part of a parenchymatous rat liver cell. The nucleus lies in the left of the field and a short stretch of the cell membrane in the bottom right hand corner. The cytoplasm is filled with several stacks of rough surfaced cisternae of the endoplasmic reticulum in parallel array together with numerous mitochondria. Microbodies with dense excentric nucleoides can also be seen as well as smaller dense lysozomes. x 10,000. (*Electron micrograph by courtesy of M. A. Epstein & R. M. Hicks, Bland Sutton Institute of Pathology, The Middlesex Hospital Medical School*).

particular tissue. Thus pancreatic cells synthesize insulin, ribonuclease and many enzymes, such as trypsin and chymotrypsin, which catalyze the breakdown of proteins. The cells of the mammary gland synthesize the milk proteins such as casein and β-lactoglobulin, and the liver cells the plasma proteins apart from γ-globulin. Thus each particular type of cell is capable of synthesizing a protein with a specific structure. The ultimate objective is to find out how this is done; the first step being to determine where within the cell the activity is centred.

Intracellular Structure

Our knowledge of the fine structure of the cell had to await the development of the electron microscope. The reason for this is that the limit of resolution of the light microscope is 200 mμ (1 mμ = 1 millionth of a mm.) whereas that of the electron microscope is 1 mμ.

Even then special methods of preparing ultra-thin sections had to be devised. Much of the interpretation in terms of cell morphology of the electron micrographs obtained has been due to G. E. Palade at the Rockefeller Institute, New York.

In Fig. 2 is shown an electron micrograph of a parenchymatous liver cell. The cell, which is surrounded by the cell membrane, consists of two parts, the nucleus and the cytoplasm. In the nucleus can be seen in some sections, but not in this figure, the nucleolus. The cytoplasm contains many structures. The largest particles are the mitochondria which can usually be recognized by the characteristic folding of the inner membrane to form cristae. As this is a liver cell one may also observe glycogen granules, these being the form in which carbohydrate is stored in the liver. Other structures are the micro bodies which contain enzymes such as catalase and the lysozomes which were first described by the Belgian biochemist C. de Duve[4]. Particular attention must, however, be paid to the so-called endoplasmic reticulum which occupies most of the space in the cytoplasm. We shall return to this later.

The general plan which has arisen of the division of labour in the cell is as follows. The information concerning the exact amino acid sequence of the proteins synthesized is possessed by the genetic material which is deoxyribonucleic acid (DNA). The DNA of the cell is confined to the nucleus. Although some protein is synthesized in the nucleus most of it is synthesized in the cytoplasm. There must therefore be a link between the DNA of the nucleus and the cytoplasm. The ribonucleic acid (RNA) of the cell fulfills this function. Although most of the RNA is confined to the cytoplasm some is found in the nucleus, especially in the nucleolus. The scheme therefore is that the DNA passes on its information to the RNA in the nucleolus. This RNA then finds its way into the cytoplasm where it serves as a template for the synthesis of protein.

The problem is narrowed therefore to the site of synthesis of protein among the morphological constituents of the cytoplasm. Although the mitochondria have the ability to synthesize protein their chief function in the cell is probably to serve as the power house for the formation of high energy phosphate bonds which are the currency of energy for all the synthetic activity of the cell. It is therefore among the non-mitochondrial constituents of the cytoplasm that attention is concentrated.

Occurrence of the Endoplasmic Reticulum

In Fig. 3 is seen a higher power magnification of an electron micrograph of the cytoplasm of the liver cell. This shows that between the mitochondria is to be found the endoplasmic reticulum (as seen in Fig. 2) but it is now clear that the outer surface of the reticulum is covered with electron-dense particles. Such particles also occur in the spaces between the reticulum. This particular form of endoplasmic reticulum is called 'rough' because of the presence of the particles. Other reticulum is found in the cell without particles and this is said to be 'smooth' (e.g. Golgi apparatus).

If a survey is made of the fine structure of a wide variety of cell types rough endoplasmic reticulum of the kind just described is often found. The apocrine cells of the pancreas of the guinea-pig are a particularly good example of this. There are, however, some cells that are relatively free from reticulum but nevertheless possess electron-dense particles.

Before the reason underlying the distribution of the rough endoplasmic reticulum among cell types can be understood, it is necessary to consider the nature of the protein being synthesized in any particular cell. All enzymes are proteins and for the functions of the cell a good supply of such protein must be available. Moreover tissues grow and protein is required for such growth. Hence much of the protein synthesized by a cell is required for internal purposes.

In contrast, some cells synthesize protein which is to be used by other parts of the body or by the young. Thus the mammary gland is synthesizing protein required in the milk and much of the effort of the liver must be directed to the synthesis of the plasma proteins, of which serum albumin is the major constituent. Such proteins are made for export from the cell. In general it is found that in cells in which, among their various activities, the synthesis of protein for export is important, there is to be found much rough endoplasmic reticulum. In other cells such as the reticulocytes, which synthesize haemoglobin but do not export it, or tumour cells which are growing but not exporting protein, there is relatively little rough endoplasmic reticulum.

It follows then that from morphological evidence the rough endoplasmic reticulum would be expected to play a key role in protein synthesis. It is necessary now to see whether the biochemical evidence supports this contention.

Biochemical Approach

One of the aims of the biochemist is to explain in terms of chemistry how proteins are synthesized by living tissues. In order to do this it would be a great advantage if a mixture of soluble substances could be found, which, when incubated together, synthesized protein. The reason for the search for soluble substances is that only in this case is it likely that the substances involved will be capable of being chemically characterized. Such an objective is not yet within sight of achievement so that the aim is at present more limited. This is to obtain from the cell morphological components which, when incubated outside the cell, will synthesize protein. Such morphological components should, of course, be as simple in structure as possible. Considerable progress towards this objective has been made.

The study of protein synthesis by isolated intracellular components not only helps in the understanding of the mechanism of protein synthesis in chemical terms, but also enables one to build up a picture of how the various components of the cell work together

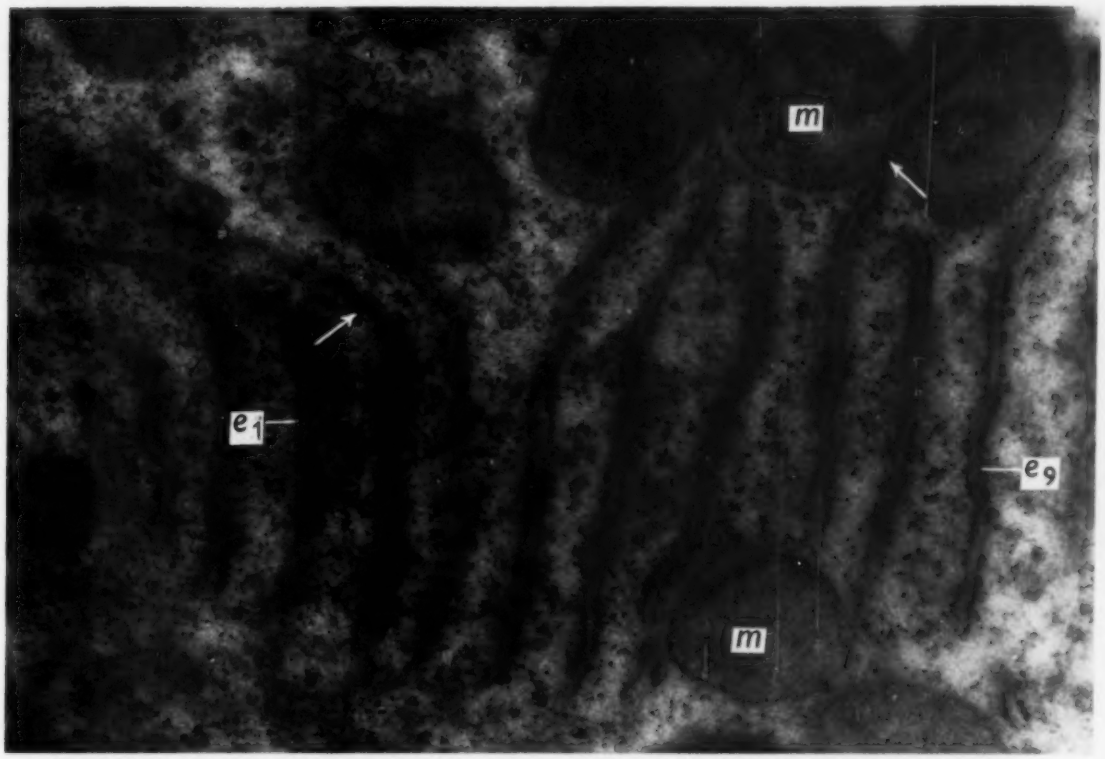


Fig. 3. Electron micrograph of a parenchymatous liver cell. An array of nine elongated profiles (e_1, e_9) of the rough-surfaced variety and five mitochondrial profiles (m) in the cytoplasm of a parenchymatous rat liver cell. The elongated profiles are disposed parallel to one another at more or less regular intervals. In three dimensions the array corresponds to a pile of vesicles. The outside surface of the membrane limiting these profiles bears numerous attached particles of small size and high density. A few similar particles appear freely scattered in the intervening cytoplasm. The arrows indicate continuity between rough-surfaced and smooth-surfaced profiles. x 49,000. (*Electron micrograph by courtesy of G. E. Palade, Rockefeller Institute, New York, ref.* 6).

first to synthesize protein and if necessary to export it.

The first problem is how to disrupt the cell and separate the morphological components. Many methods are available for this purpose, but the one selected is invariably that which in the particular circumstances will do least harm to the components once the cell membrane has been broken. With liver the method of choice is a 'glass homogenizer' in which a glass or Teflon pestle rotates in a glass mortar, the pestle being moved slowly up and down vertically. After such a process the 'homogenate' must be fractionated to separate the components. Again there are several methods of approach but the most common is to make use of differences in density by centrifuging at progressively increasing speeds, or in media of different densities. This is illustrated in Fig. 4. There is no difficulty in obtaining the 'nuclear' and 'mitochondrial' fractions but the microsomal fraction is best obtained by the use of a preparative ultracentrifuge, for very high centrifugal forces are required. At all stages of the process it is necessary to keep the temperature near to freezing point to preserve the integrity of the structures.

Use of Radioactive Amino Acids

Having obtained the different subcellular fractions it is necessary to determine which is most active with respect to protein synthesis. As proteins are such large and complex molecules it is difficult to demonstrate their synthesis and it is impossible to conceive how the subject could have advanced if it were not for the availability of radioactive amino acids. These are usually 'labelled' with either carbon-14 (^{14}C), sulphur-35 (^{35}S) or tritium (^{3}H). If a protein is synthesized in the presence of a radioactive amino acid the protein will become radioactive.

If a rat be injected with ^{14}C-amino acid and pieces of liver removed, at different times after injection, homogenized and fractionated, then by determining the radioactivity of the protein in the different fractions it is possible to find out which fraction is most active in protein synthesis. Fig. 5 shows the results of such an

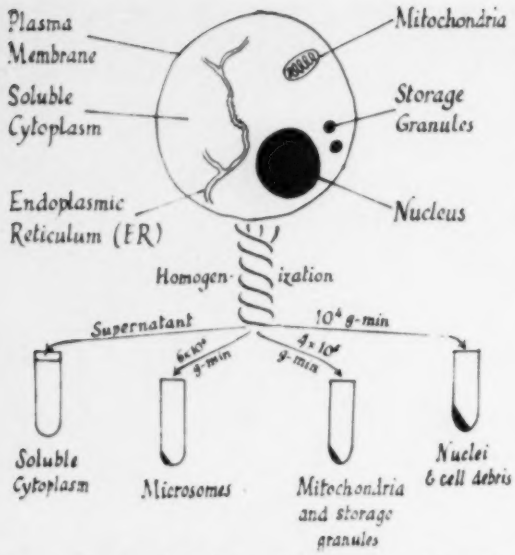


Fig. 4. Fractionation of subcellular particles. The units quoted as *g* min. represent the centrifugal force *g* multiplied by the number of minutes the material was submitted to this force.

experiment from which it is seen that the microsome fraction is the most active. It was experiments such as this by Zamecnik and his colleagues[5] that directed attention to the microsome fraction. The next problem was to find out the nature of this fraction.

Chemical analysis showed that of all the fractions isolated by centrifugation the microsomes contained the highest ratio of ribonucleic acid to protein. This was interesting in view of the suspicion that ribonucleic acids played an important role in protein synthesis.

Morphological Structure of the Microsome Fraction

Our knowledge of the morphological structures that go to make up the microsome fraction has been derived mainly from the work of Palade and Siekevitz at the Rockefeller Institute, New York. They have shown that with rat liver[6] and guinea-pig pancreas[7], in spite of the presence of a wide variety of components, the predominant structures present are pieces of the rough surfaced reticulum. This finding, of course, fits in with the previous observations leading to the conclusion that this component plays an important role in protein synthesis.

Activity of Isolated Components

Zamecnik and Keller[8] first showed how to incubate the microsome fraction of rat liver with ^{14}C-amino acids and obtain radioactive protein. It is of interest to note that for this process to occur there had to be present some of the soluble cytoplasm, although this was itself inactive. In these experiments the incorporation of ^{14}C-amino acid into the mixture of protein-like

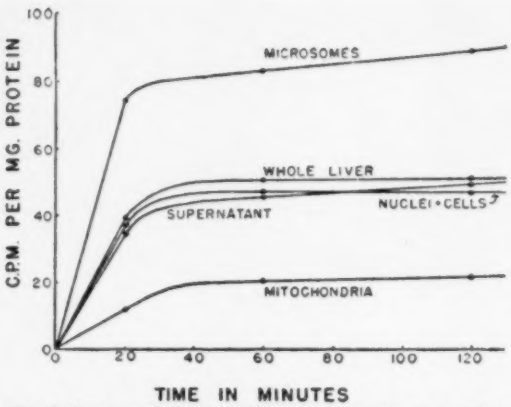


Fig. 5. Incorporation of [^{14}C] leucine into rat liver proteins. Time curve for the incorporation of [^{14}C] leucine into proteins of liver cell fractions *in vivo*. Liver samples, each approximately 10 per cent of the total liver, were removed at 20 min. and 1 hr. (ref. 5).

substances, precipitated with an agent such as trichloroacetic acid, was studied. Each cell type is capable of the synthesis of a particular well-characterized protein, and so the question is asked whether the component of the liver cell represented by the microsome fraction can synthesize serum albumin, because it is the synthesis of this protein which is particularly characteristic of the intact liver cell. We have studied this for some years and all the evidence that has been adduced is consistent with such a synthesis. The isolated microsome fraction from rat liver is therefore a most useful tool for the biochemist.

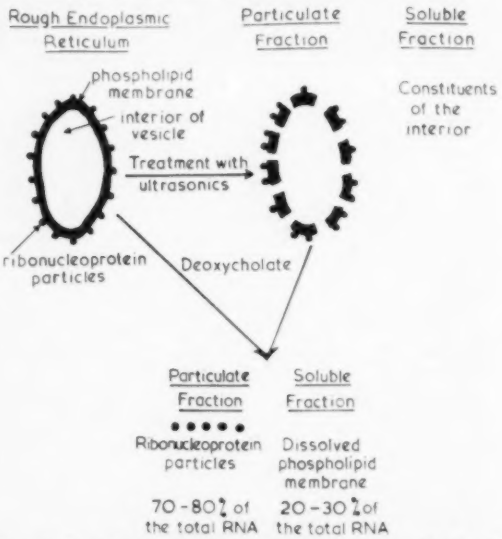


Fig. 6. A diagrammatic representation of the effects of various treatments on the rough endoplasmic reticulum of rat liver.

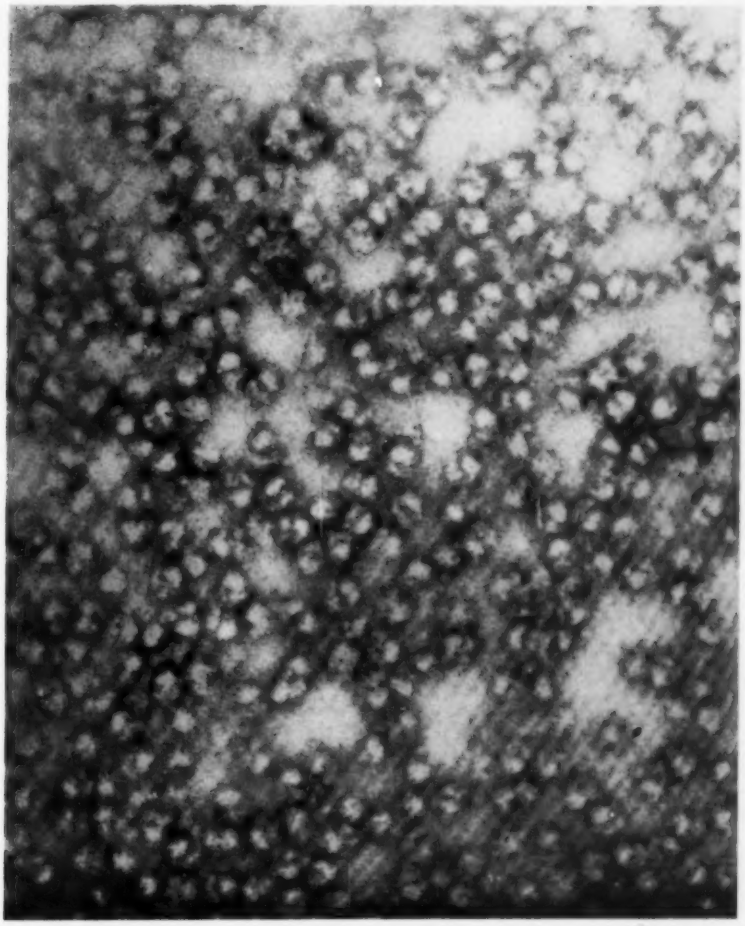


Fig. 7. Electron micrograph of ribosomes from E. coli. The particles were negatively stained with phosphotungstic acid. Most of the particles show two unequal subunits. x 200,000. (*Electron micrograph by courtesy of H. E. Huxley, Biophysics Department, University College, London*).

Nature of the Rough Surfaced Endoplasmic Reticulum

Palade and Siekevitz found that the membrane component of the rough endoplasmic reticulum present in the microsome fraction was osmotically sensitive. In our studies on the synthesis of serum albumin we found that the appearance of the microsome suspension could be markedly altered by the application of ultrasonic vibrations. For this purpose we used an ultrasonic drill (available from M.S.E. Ltd., Spenser Street, London, S.W.1). Associated with the clearing of the suspension was the release of a considerable quantity of serum albumin in a soluble form. We believe that the ultrasonic vibrations disrupt the membranes and release their soluble constituents as shown in Fig. 6.

While the isolation of fragments of the rough endoplasmic reticulum represents a step in the direction of morphological simplicity compared with the whole cell it may be asked whether an even simpler system might not be devised.

Two groups in America found some years ago that if the microsome suspension be treated with the bile acid, deoxycholic acid, there was a very marked clearing of the suspension. If however the apparently clear preparation be subjected to centrifugation for a long time a small pellet was obtained. Chemical analysis showed the material to contain approximately equal parts of ribonucleic acid and protein and hence it is said to consist of ribonucleoprotein.

When the ribonucleoprotein was examined under the electron microscope it was found to consist of small (about 15 mμ) electron dense particles which were approximately the same size as those attached to the rough endoplasmic reticulum. The conclusion was that the deoxycholate had broken up the membrane which,

at least in the liver, is rich in phospholipids leaving only the electron dense particles. Thus the particles in the reticulum are probably composed of ribonucleoprotein.

Metabolic Activity of Ribonucleoprotein Particles

When Zamecnik and his colleagues[9] did an experiment similar to that previously described in Fig. 5, but this time sub-fractionating the microsome pellet by the use of deoxycholate, evidence was obtained that the initial site of incorporation of ^{14}C-amino acid in the intact liver was the ribonucleoprotein particles. It was only later that the radioactive protein passed to the membrane component of the endoplasmic reticulum.

At that time (1955) experiments designed to obtain ribonucleoprotein particles which synthesized protein, when incubated, failed. However conditions have now been found under which the ribonucleoprotein particles will incorporate ^{14}C-amino acid into protein. Moreover we now believe them to be capable of the synthesis of serum albumin.

As mentioned previously the reticulocyte cell does not appear to contain rough surfaced endoplasmic reticulum, the electron dense particles occurring free in the cytoplasm. Such particles on isolation are also able to effect the synthesis of protein. Schweet and his colleagues[10] have demonstrated the synthesis of haemoglobin by such particles.

Ribonucleoprotein Particles from Bacteria

Bacteria differ from most types of living cell in that they do not possess a clearly defined nucleus. It is however possible to detect in them electron dense particles, although not attached to endoplasmic reticulum, and to isolate the particles after disrupting the cells.

Again it is found that the particles consist of ribonucleoprotein and they have therefore become known as ribosomes. The fine structure of the ribosomes from *Escherichia coli* has been studied by H. E. Huxley and G. Zubay[11]. For this they have used the negative staining technique with phosphotungstic acid first described by C. E. Hall[12]. In this method the material is sprayed with phosphotungstic acid and then dried. As it dries the solution retracts from morphologically smooth areas but remains round the edge of the particles and in any grooves in the structure. The results obtained are shown in Fig. 7. This shows that the ribosomes are composed of two unequal parts.

Direct evidence of the role of the ribosomes in protein synthesis has been slow to obtain but recently such evidence has been forthcoming.

Conclusions

Although the story is as yet incomplete the combined efforts of electron microscopists and biochemists have enabled them to present a working hypothesis of where proteins are synthesized and the structures involved in their export from the cell. The units which play the leading role appear to be the ribonucleoprotein particles which are to be found in virtually all living cells. Much effort is being devoted to the detailed study of exactly how the amino acids are linked together by such particles. The biochemist now has a tool which is not too far short of his objective in terms of morphological simplicity.

Although the work described on the synthesis of protein is probably the first and certainly the most dramatic fruit of the collaboration between biochemists and electron microscopists it is not unique. Thus studies are now in hand on glycogen synthesis, electron transport in mitochondria and the absorption of lipid through the wall of the intestine, to mention only a few. It is certain that we may expect many such developments in the near future.

REFERENCES

[1]Campbell, P. N. (1960). Biol. Rev., **35**, 413.
[2]Sanger, F. and Smith. L. F. (1957). Endeavour, **16**, 48.
[3]Ingram, V. M. (1957). Nature, Lond., **180**, 326.
[4]deDuve, C. (1959). In Subcellular Particles, Ronald Press Co., N.Y.
[5]Keller, E. B., Zamecnik, P. C. and Loftfield, R. B. (1954). J. Histochem. Cytochem., **2**, 378.
[6]Palade, G. E. and Siekevitz, P. (1956). J. Biophys, Biochem. Cytol., **2**, 171.
[7]Palade, G. E. and Siekevitz, P. (1956). J. Biophys. Biochem. Cytol., **2**, 671.
[8]Zamecnik, P. C. and Keller, E. B. (1954). J. Biol. Chem., **209**, 337.
[9]Littlefield, J. W., Keller, E. B., Gross, J. and Zamecnik, P. C. (1955). J. Biol. Chem., **217**, 111.
[10]Schweet, R. Lamfrom, H. and Allen, E. (1958). Proc. Nat. Acad. Sci. Wash., **44**, 1029.
[11]Huxley, H. E. and Zubay, G. (1960). J. Mol. Biol., **2**, 10.
[12]Hall, C. E. (1955). J. Biophys. Biochem. Cytol., **1**, 1.

Fleming Memorial Fund

The Trustees of the Fleming Memorial Fund for Medical Research announce that the Board will be considering the making of its first grants to assist basic medical research before the end of 1961.

Applications should be sent to:
The Secretary to the Trustees,
The Fleming Memorial Fund for Medical Research,
26 Upper Brook Street,
London, W.1.

The Fund was launched in March this year, together with its 1961 British and international appeal for money. The first Board of Trustees consists of Lord Heyworth (Chairman), Sir Lindor Brown, Viscount Crookshank, Sir Henry Hancock, Sir Hans Krebs, Prof. P. B. Medawar and Lord Sinclair of Cleeve. Sir Charles Dodds is Chairman of the Fund's Scientific Advisory Committee.

The Fund exists to commemorate Sir Alexander Fleming, and his discovery of penicillin, by raising money to provide assistance for basic medical research anywhere in the world. This assistance may be given by grants to universities, research institutions and others engaged in basic medical research, and by way of national and international fellowships tenable at universities and research institutions. Full details were contained in the special issue of *Laboratory Practice* devoted to the Fleming Memorial Fund (April, 1961).

The Trustees are empowered to administer both the capital and income of moneys raised and to seek advice wherever and whenever they feel it necessary in making grants, having particular though not exclusive regard for the needs of certain institutions which were concerned in the discovery and development of penicillin.

Contributions to the Fund already notified amount to a gross value of some £350,000, including covenanted donations. Regional appeals are being organized in this country and overseas, in order substantially to increase this figure

RECENT ADVANCES

IN

FORENSIC SCIENCE

by L. C. Nickolls

Director, Metropolitan Police Laboratory, London, S.W.1

FORENSIC science is a comparatively recent science and is therefore advancing rapidly and continuously in all its branches.

It has been a hard and difficult task adapting past knowledge and experience to the needs of this new science. It has been an additional necessity that modern scientific discoveries must also be integrated into forensic science. I will endeavour to show how we are succeeding in the task.

As this lecture is being given in connection with an exhibition of instruments, I propose to confine my remarks largely to the impact of instrumentation on forensic science.

In this respect, however, I should like to draw your attention to the remarks made by Dr. Chirnside in his Presidential Address to the Society for Analytical Chemistry, when he said that all science, especially quantitative science, depends on instruments. Modern instrumentation does not differ materially from the old in its scientific value—only in its cost. But just as the classical instruments, when first invented, opened new realms of scientific investigation, so modern instruments are doing the same at the present time. It is possible to make examinations and obtain results with modern instruments which were previously impossible and could not even be envisaged.

In order to appreciate the position of instruments in forensic science, it is necessary to consider what is the work of a forensic science laboratory. It is the duty of the forensic scientist to examine any material recovered either by the police or by himself in connection with a crime which is regarded as having some bearing on the crime and concerning which further information is required. This, of course, is a very wide duty embracing many different types of examination and it may be thought to be too wide to be encompassed. However, by good team work there are few problems which cannot be solved and which require outside help, although when such cases arise we always seek such assistance and receive great consideration, advice and help from our specialist colleagues on these occasions.

If we consider the work of the laboratory we find that it can be classified into two main groups, firstly examination of materials directly associated with a particular type of crime, such as for example blood-staining in crimes of violence, and secondly examination of materials which are associated with the scene of a crime or with the criminal. Concerning the first group, fortunately the categories of crime committed are limited and criminals tend to commit them in similar manners. Accordingly we make identical examinations as a routine matter many times every year. With the second group, however, we are liable to encounter any substance which is found in use in the daily life of the community. It will be obvious that while it will be economic to purchase instruments to assist in the first group of examinations, it probably will not be economic to purchase specialized instruments to examine the varied and often only rarely received materials of the second group.

When considering whether an instrument, usually a very expensive instrument, should be purchased, the forensic scientist has to bear in mind a number of facts. The most important factor in his work is accuracy and

certainty. Time and labour are secondary factors which must never affect the first. Because of this, classical methods of examination, when they can be employed, are normally used because of their established degree of accuracy and certainty of identification. It is only when other methods must be used to achieve our object that the need of instrumentation is considered. Science has progressed so fast of recent years, and the needs of the forensic scientist with it, that notwithstanding his adherence to classical methods he has been forced to make use increasingly of modern instrumentation. His only regret is that manufacturers do not make instruments for his especial needs because the demand for them is naturally very limited and the cost would probably be prohibitive.

Let us consider how the above considerations affect the work of the various departments.

Biological Laboratory

In this laboratory are examined all materials derived from living matter. This work is necessarily largely observational and has not been greatly affected by modern instrumentation. The work has been greatly assisted, however, by improvements in microscope design. The modern microscope is more stable in operation and has better definition than older models. Although we rarely use powers as high as 1/12th, speed and accuracy of work are greatly increased by this stability and definition.

Comparison microscopes have not improved, for our purposes, to the same extent possibly because of difficulties of design and the small demand. The two main faults we find in the present instruments are that the lighting on both fields is rarely exact in brilliance and colour while the fields of view never seem as large as we would like. Much of the work in this laboratory owes its advancement to improvements in technique because instrumentation does not come into the work. For example, in crimes of violence, which are very common at present, blood staining is the most frequent material for examination. The examination of blood stains is entirely a question of techniques. For some blood examinations we use electrophoresis but the occasions for this are rare. For example, electrophoresis is used to separate the varieties of haemoglobin present in different races of human beings, and it can be used to identify foetal haemoglobin in foetal blood. The difference between adult and foetal haemoglobin on electrophoresis is not large. The two haemoglobins can be identified more simply by other means even in admixture. Adult haemoglobin is rapidly destroyed by dilute alkalis losing its pink colour and becoming pale greenish yellow. Foetal haemoglobin is much more resistant to destruction. The change can be plotted against time by measuring the transmittance of a solution in $\frac{N}{25}$ NaOH solution in a spectrophotometer at 5,400 Å, the wavelength of the α haemoglobin band. (Fig. 1).

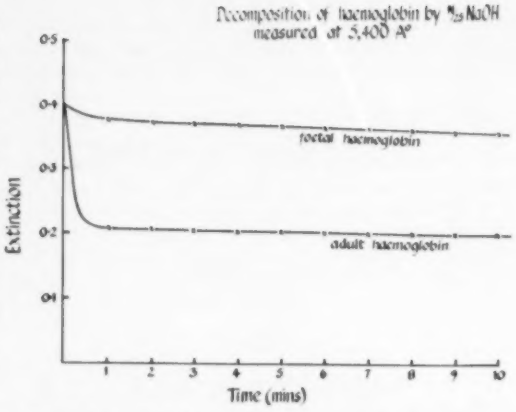


Fig. 1. Graph showing the rapid decrease in absorption of normal blood in alkaline solution as compared with foetal blood.

Chemical and Physico-chemical Laboratory

It is this laboratory in which we have found modern instrumentation to be of most value and in which the bulk of our modern instruments is installed. Although we use these instruments, care must always be taken not to attribute to the results more information than is really conveyed. For example, the spectrophotometer is an established instrument in the laboratory. Sometimes the results obtained give complete identification, sometimes they do not. The police use anthracene as a marker in certain circumstances and we get clothing and other articles submitted to us in order to identify anthracene on them. At times the amount of anthracene may be small and identification by classical methods is difficult or impossible. The U.V. spectrum of anthracene (Fig. 2) is not only unique but is very sensitive and by this means minute quantities of anthracene can be conclusively identified. On the other hand, barbiturates have a characteristic band at 240 Å in alkaline solution and no band in acid solution (Fig. 3). This band can be used to determine the amount of barbiturate present but does not, in my opinion, identify the material conclusively as a barbiturate. This should be done in some other manner.

While the work of this laboratory is naturally very varied, quite a number of standard techniques are used and adapted to many different types of examination. To illustrate what has been achieved by the application of modern methods, I will describe the normal routine search for a possible poison and we can see clearly how this has been revolutionized by modern instrumentation. When a poison is suspected in the organs of a deceased person or an article of food, etc., it is necessary to identify some poisons individually but we can look for certain poisons as groups of similar chemical substances as follows:

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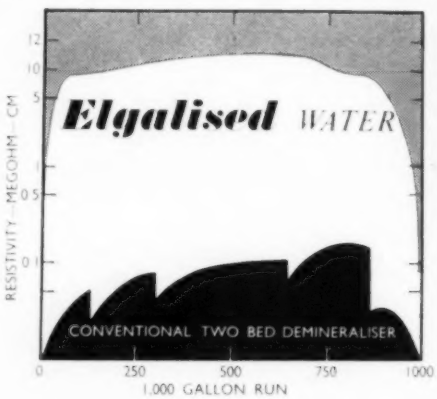

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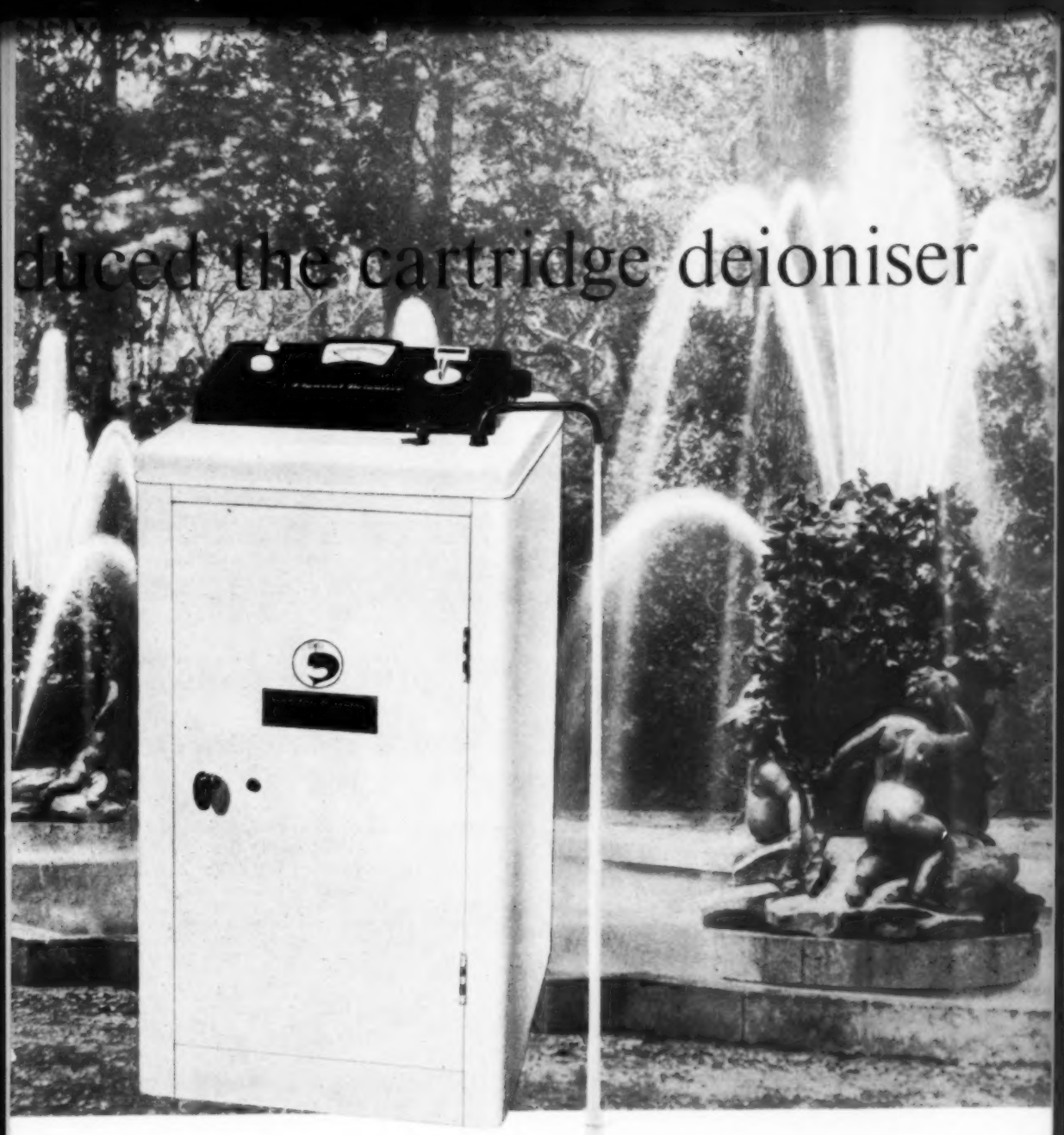

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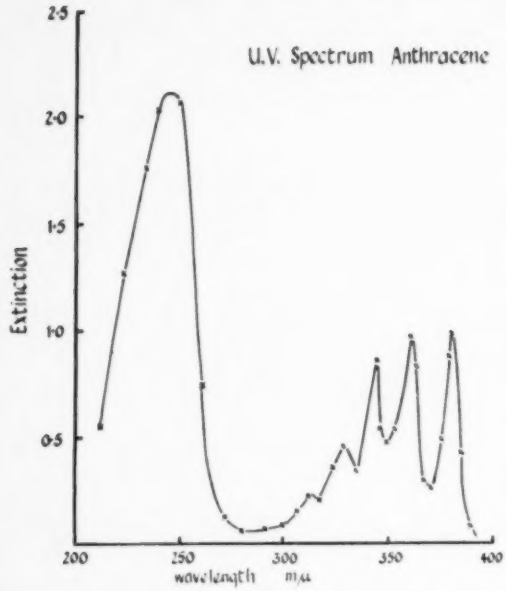


Fig. 2. U.V. Spectrum of anthracene. This spectrum shows the characteristic absorption bands of anthracene.

(i) *Metallic poisons.*—In former times these were sought by classical methods which were lengthy and not very delicate. Now we separate the metallic constituents by suitable methods and are the residue on a quartz spectrograph. Amounts of metals varying from 0·1 to 10 mcg. depending on the metal can be positively identified, this being well below poisonous or even accidental amounts. (Fig. 4). Quantitative determination of any metal identified is still made by classical methods.

(ii) *Organic poisons* (acidic).—It must be borne in mind that the concentration of organic poison in fatal poisoning cases in the organs rarely reaches 100 parts per million and may be a thousand times less than this, while in the case of sub-lethal doses very much smaller concentrations are encountered. The organic poisons are therefore extracted by solvents together with considerable amounts of extraneous matter. The poison is extracted in acid solution to obtain the acid fraction which is normally aspirin or a barbiturate. Aspirin is readily identified. The barbiturate is chromatographed (Fig. 5) and by this means a preliminary identification is made. The concentration is determined

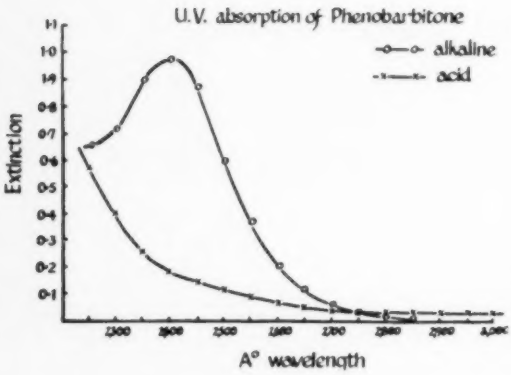


Fig. 3. U.V. spectrum of phenobarbitone, showing an absorption band in alkaline solution which is not present in acid solution.

Fig. 4. (*Below*). **Suspect murder by arsenic. Portion of the line spectra showing the sensitive lines of arsenic at 2,288, 2,349, 2,369-70 and 2,456 Å. The spectra in order from top to bottom are as follows: (1) Iron; (2) Arsenic control; (3) Reinsch test on liver arcing the copper foil; (4) Heavy metals precipitated from liver digest; (5) R. U. powder.**

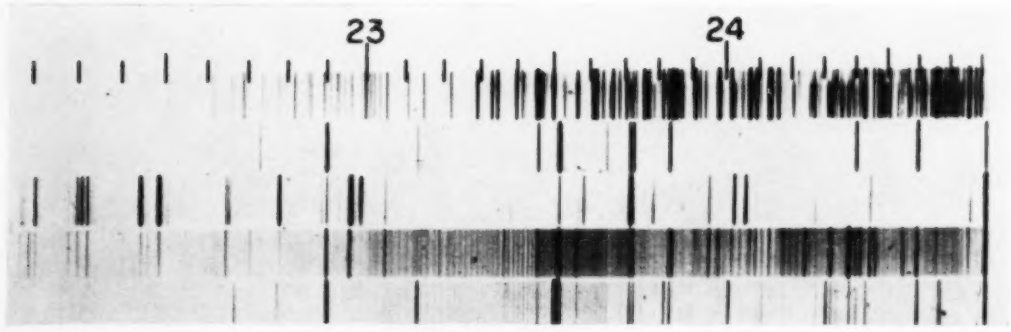


Fig. 5. Chromatogram of certain of the barbiturates developed with cobalt nitrate and ammonia vapour, showing the varying R.F. values.

by the acid/alkaline peak at 240 mμ in the U.V. spectrophotometer (Fig. 3) while final identification is made wherever possible by the x-ray diffraction pattern (Fig. 6).

(iii) *Organic poisons* (neutral).—This is an increasingly important class of poisons, which includes straight chain ureides such as carbromal, and they are somewhat difficult to characterize if they refuse to crystallize. Recently, however, we have devised a good method using reverse phase chromatography (Fig. 7).

Not only are many of these substances very recent with little known about their chemistry but, in addition, their structure is such that they are often not readily identifiable.

(iv) *Organic poisons* (alkaline).—The number of these poisons increases daily. The natural alkaloids are rarely met in practice compared with the synthetic alkaloids, the tranquillizers, antihistamines and other alkaline drugs so commonly prescribed. Thus identification and characterization are by no means a simple straightforward operation. The presence of such a drug is found by standard chemical alkaloidal tests. This is confirmed by chromatography, spraying the paper with iodo-

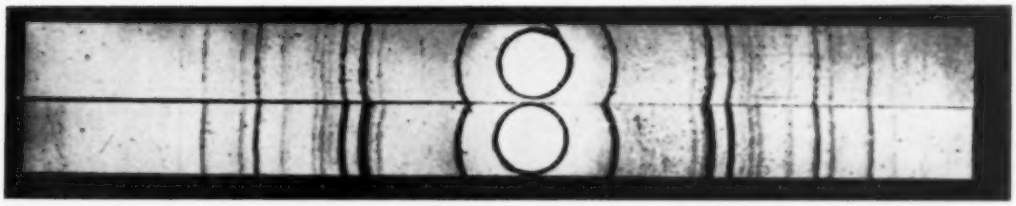


Fig. 6. X-ray diffraction pattern of lethal dose of barbiturate recovered during routine examination of organs in an unexplained death. *Top:* **Material recovered from organs;** *Bottom:* **Control phemitone.**

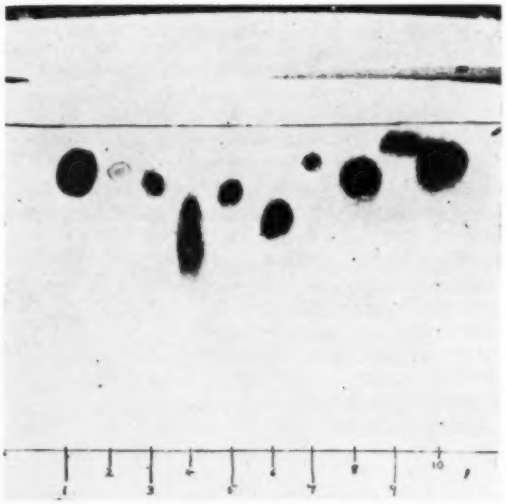


Fig. 7. Reverse phase chromatogram of a series of neutral drugs developed by chlorination, followed by starch K.I.

platinic acid solution. It must be remembered that this only detects tertiary amines; ephedrine, benzedrene and caffeine for example have to be detected separately. As these alkaloidal residues are rarely crystalline, the best methods of characterization are the U.V. absorption spectra and the I.R. spectra.

From what I have just described you will appreciate that the pre-war forensic toxicologist coming into a modern laboratory would find himself surrounded by a host of drugs completely unknown to him being examined by methods which would seem very quick though strange and using instruments of quite novel design and conception.

Toxicology is, however, only one of the many occupations of the chemical laboratory. A combination of instrumental, classical and special methods adapted to our particular problems are often combined to obtain a result. When fluids have to be examined the vapour phase chromatograph is a necessity. We find that identifications which were impossible, owing either to complexity or to smallness of sample, a few years ago can now be readily performed by the use of the vapour phase chromatograph.

An example of the first type is shown in cases of larceny of petrol. It is necessary in such cases to show that a number of samples of petrol are identical. This is an extremely difficult task when the normal methods of examination are employed. By vapour phase chromatography, however, a complete and accurate picture of the composition of the petrols can be quickly obtained.

An example of the second type is that of soap. For certain reasons it may be necessary to identify soap in small proportions in body fluids. Soap is normally a complex mixture of the sodium salts of higher fatty acids and it does not lend itself to accurate determination. We have found that the fatty acids from not less than 10 mg. of soap present in up to 20 ml. of blood, i.e. ·05 per cent soap in solution, can be converted into the methyl esters and then separated and identified by vapour phase chromatography so accurately that the source of the soap can be discovered (Fig. 8).

Special Laboratory

In this laboratory are performed a number of examinations which are special to a forensic science laboratory and are rarely encountered elsewhere. These include the examination of firearms, bullets and cartridges, of scratch marks and instrument marks and footprints.

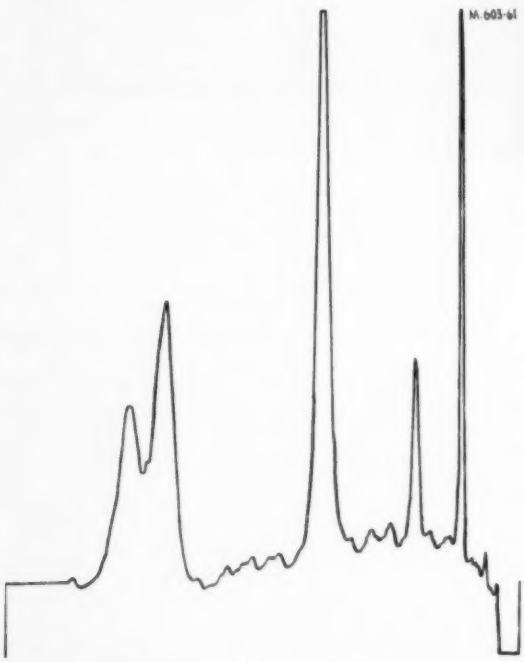
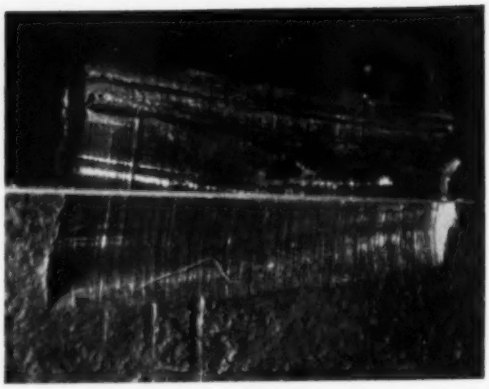


Fig. 8. V.P. chromatogram of methyl esters of fatty acid fraction of body fluid, showing characteristic peaks due to soap fatty acids.



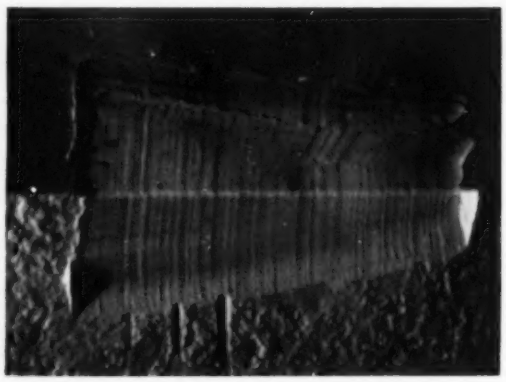


Fig. 9. Photograph showing comparison of screwdriver marks on lock compared with control. *Top:* **in its natural state;** *Bottom:* **after smoking with magnesium oxide.**

Naturally, there is little in the way of instrumentation to assist us in these fields. The comparison microscope is today essentially the same as it was before the war with the same defects as already described. The most notable advance has been an advance in technique of American origin. This technique consists in exposing all scratch marks to the smoke of burning magnesium ribbon until a thin layer of oxide has been deposited on the surface. The resulting smoked scratch marks are infinitely easier to examine and compare than the original marks (Fig. 9).

These descriptions that I have given do not cover more than a few of the aspects of forensic science but they will show, I hope, that the forensic scientist is aware of the advances of sciences and, wherever possible, endeavours to adapt them for his own particular purposes.

ARTICLES

THE FUNDAMENTAL ASPECTS OF GAS CHROMATOGRAPHY

by M. A. Khan, B.Sc. (Pb), M.Sc. (Alig.), Ph.D. (Cantab.)

'Shell' Research Limited, Thornton Research Centre, P.O. Box 1, Chester

PART II—A (GENERAL THEORY)

IN Part I, we outlined the theory which describes the behaviour of a single chromatographic band during the course of its development. For linear distribution isotherms, we derived from the theory that the band travels through the column with a constant width and speed. In other words, the band moves with sharply defined front and rear boundaries, does not broaden on its way down the column, and hence the shape of the elution curve* is rectangular. For non-linear isotherms, however, the situation was shown to be relatively complex. It was proved that if the isotherm is concave to the concentration axis (c-axis) so that the distribution ratio $\left(\frac{C}{c} = \frac{f(c)}{c}\right)$ decreases with the increase in c, the band has a sharp boundary followed by a long tail. On the other hand, if the isotherm is convex to the c-axis so that the distribution ratio increases with the increase in c, the shape of the band is reversed i.e. the rear edge is sharp while the front has a projection of gradually decreasing concentration (Part I, Fig. 5, 6).

The theory outlined correctly describes the movement of the centre of gravity of a band through the column. However, it does not explain why the band gets wider and its boundaries become less defined as it passes down the column. Even with linear isotherms the shape of the elution curve is changed, it no longer remains rectangular but assumes the shape of a normal curve of error†.

We shall remind ourselves of the two main assumptions on which the theory was based:

(i) at any given point of the column, equilibrium between the solute concentrations in the two phases is reached practically instantaneously.

(ii) diffusion of solute in the mobile phase along the axis of the column is negligible.

Non-Instantaneous Attainment of Equilibrium

When rate constants for sorption and desorption processes are not infinitely large, equilibrium between the two phases is not reached instantaneously. In more popular language this is described as a phenomenon of resistance-to-mass transfer, and the mechanism by means of which solute is transported from one phase to another is usually assumed to be a diffusional process. In a chromatographic column, the stationary phase* sorbs solute from a stream of the mobile phase flowing through the void volume between the solid particles; the solute molecules must diffuse through the interstices in order to reach the surface of the stationary phase. Similarly, the solute has to diffuse from the interior of the stationary phase in order to get back in the mobile phase. If the sorption and desorption proceed at low rates, some molecules would get relatively far ahead or lag behind giving rise to a spread in the front as well as in the rear boundary of the band. The overall effect of non-equilibrium conditions or finite rate constants is, therefore, to 'smear out' the band.

Longitudinal Diffusion

The longitudinal diffusion, when referred to the spreading of the chromatographic band, includes both true longitudinal molecular diffusion and apparent longitudinal diffusion†. The true molecular diffusion occurs because of the concentration gradients existing within the mobile phase along the length of the column, whereas apparent diffusion comes into play because of the multitude of zigzag paths of unequal lengths and widths. We can offer a simple physical picture of apparent diffusion if we assume the pore space within the packed bed to be equivalent to a bundle of parallel capillaries of different diameters but of the same length. Portions of solute which enter the cross-section of a given capillary at the same time will travel through it at different velocities; those moving along the capillary axis will be the fastest and come out first. Also, some portions of solute might flow through wide capillaries while others might flow through narrow ones. According to Poiseuille's law, material in each capillary will be flowing at different rates. As a result of all this, some portions of solute may get ahead while others may lag behind with respect to the mean velocity of flow.

EFFECT OF DISTURBING FACTORS‡ ON BAND WIDTH

Band width is a factor of paramount importance

*An elution curve represents a plot of the concentration of solute emerging from the column exit against the total volume of the mobile phase passed through the column. It is sometimes defined as the plot of concentration of solute in the mobile phase against the distance measured from the column top.

†Also known as the Gaussian curve, normal distribution curve or probability curve.

*As in Part I, the stationary phase may be the solid particle itself or a liquid film supported on the particle.

†In the literature the term 'eddy diffusion' is used instead of apparent diffusion. The term 'eddy diffusion' is rather misleading, especially when the fluid flow through a packed column is well below the critical Reynolds number calculated on particle size and there is no obvious possibility for turbulent flow. Justification for the use of apparent diffusion will soon be clear.

‡All those factors, such as diffusion, non-attainment of equilibrium etc. which cause 'band broadening' or 'band dispersion' will be named as disturbing factors.

because it gives a direct measure of the 'peak sharpness'* and on it rests the fundamental concept of 'column efficiency'.† There are basically two different theories which deal with the effect of disturbing factors on band width either implicitly or explicitly. These are commonly known as the plate theory and the rate theory.

(a) Plate Theory

The theoretical plate concept was initially introduced by Peters[10] in connection with the performance of distillation columns. Martin and Synge[11], however, were the first to apply this concept to a partition chromatographic column. The plate theory assumes the column length to be divided into a large number of adjacent layers commonly known as theoretical plates. The thickness of each layer or Height Equivalent to a Theoretical Plate (HETP) is determined by the condition that the mean concentration of solute in the stationary phase within the layer is in equilibrium with its effluent, i.e. within each plate there is perfect equilibrium between the two phases. The behaviour of the plate column is calculated on the assumption that the distribution isotherm is linear and the distribution ratio (partition coefficient) remains unaffected by the presence of other solutes. This ingenious device of dividing the column into theoretical plates takes into account the effect of diffusion and non-attainment of equilibrium rather implicitly. It is understandable that for very small values of HETP the diffusion of solute in the mobile phase from one plate to another can be neglected. Also for a column of an infinitely large number of plates (HETP = o), the effect of non-attainment of equilibrium will be nil and the band width will be completely determined by the sample size. This implies that HETP is a convenient measure for expressing the magnitude of disturbing factors. The results derived from the plate theory show that when a solute initially placed on one theoretical plate has travelled a distance of n theoretical plates down the column, it is spread out into a band the relative width‡ of which, measured at $\frac{1}{\sqrt{e}}$ th of the maximum concentration, is equal to $2 \times (\sqrt{n})$. From an algebraic treatment of the plate theory, Martin and Synge derived the following expression relating the total quantity q_n of solute in plate n with the volume of the mobile phase passed through the column:

$$q_n = \frac{1}{\sqrt{2\pi n}} \exp. \left[\frac{-\left(\frac{V}{v_R} - n\right)^2}{2n} \right]$$

Or, on putting $q_n = v_R c_n$, where c_n is the concentration in the mobile phase of plate n and v_R is the retention volume per plate:

$$c_n = \frac{1}{v_R\sqrt{2\pi n}} \exp. \left[\frac{-\left(\frac{V}{v_R} - n\right)^2}{2n} \right]$$

$$= \frac{1}{(v_R\sqrt{n})\sqrt{2\pi}} \exp. \left[\frac{-(V - nv_R)^2}{2(v_R\sqrt{n})^2} \right] \quad \ldots (13)$$

This represents the equation for the normal error curve which is graphically shown in Fig. 7. It can be shown from the geometrical properties of the curve that

$$n = \left(\frac{V_R}{\sigma_1}\right)^2 \quad \ldots (14)$$

where V_R is the retention volume referring to the centre of the peak and $\sigma_1 = v_R\sqrt{n}$ is the standard deviation.* For the Gaussian curve, σ_1 corresponds to $\frac{1}{\sqrt{e}}$th of the maximum concentration of the band.

Alternatively, (14) may be written as:

$$n = 16\left(\frac{V_R}{W}\right)^2 \quad \ldots (14a)$$

where $W = 4\sigma_1$ is the band width representing the distance between the intersections of the inflexional tangents with the base line as shown in Fig. 7.

Sometimes band width at half the peak height, Δ, is used. It is clear from Fig. 7 that $W = \sqrt{\frac{2}{\ln 2}}\,\Delta$. Hence, equation (14a) may be expressed in the form:

$$n = 8\ln 2.\left(\frac{V_R}{\Delta}\right)^2$$

$$= 8 \times (2{\cdot}30 \log_{10} 2)\left(\frac{V_R}{\Delta}\right)^2$$

$$= 5{\cdot}55\left(\frac{V_R}{\Delta}\right)^2 \quad \ldots (14b)$$

*Peak sharpness is directly measured by the band width and depends upon sample size. For large sample volumes, the peak has a flat top.

†Column efficiency or HETP is independent of the sample volume.

‡The relative band-width is the ratio of the band dispersion to retention volume per plate.

*The root-mean-square deviation (RMSD) or simply the standard deviation (σ) of a function f(x) is generally defined as:

$$\sigma^2 = \int_{\circ}^{\infty} (x - \bar{x})^2 f(x)\, dx,$$ where $\bar{x}$ is the mean around which f(x) is distributed.

Putting $v_R\sqrt{n} = \sigma_t$, $V = x$, $nv_R = \bar{x}$ and $c_n = y$, equation 13 may be written as:

$$y = \frac{1}{\sigma_t\sqrt{2\pi}} \cdot e^{-\frac{(x-\bar{x})^2}{2\sigma_t^2}}$$

Evidently peak maximum occurs at $x = \bar{x}$. Hence,

$$MN = y_{max} = \frac{1}{\sigma_t\sqrt{2\pi}} = \frac{1}{v_R\sqrt{2\pi n}}$$

$$(c_n)_{max} = \frac{1}{\sqrt{2\pi n}}$$

} For unit amount of solute injected into the column

$$\frac{y}{y_{max}} = e^{-\frac{(x-\bar{x})^2}{2\sigma_t^2}}$$ (For any point on the curve).

In particular:

(i) at A: $\frac{y}{y_{max}} = \frac{1}{\sqrt{e}} = e^{-\frac{1}{2}} = e^{-\frac{(x-\bar{x})^2}{2\sigma_t^2}}$ $\therefore x = \bar{x} \pm \sigma_t$

(ii) at B: $\frac{y}{y_{max}} = \frac{1}{2} = e^{-\ln 2} = e^{-\frac{(x-\bar{x})^2}{2\sigma_t^2}}$ $\therefore x = \bar{x} \pm \sqrt{2\ln 2}\,\sigma_t$

(iii) at D: $\frac{y}{y_{max}} = \frac{1}{e} = e^{-1} = e^{-\frac{(x-\bar{x})^2}{2\sigma_t^2}}$ $\therefore x = \bar{x} \pm \sqrt{2}\,\sigma_t$

(iv) at E: $\frac{y}{y_{max}} = \frac{1}{e^2} = e^{-2} = e^{-\frac{(x-\bar{x})^2}{2\sigma_t^2}}$ $\therefore x = \bar{x} \pm 2\sigma_t$

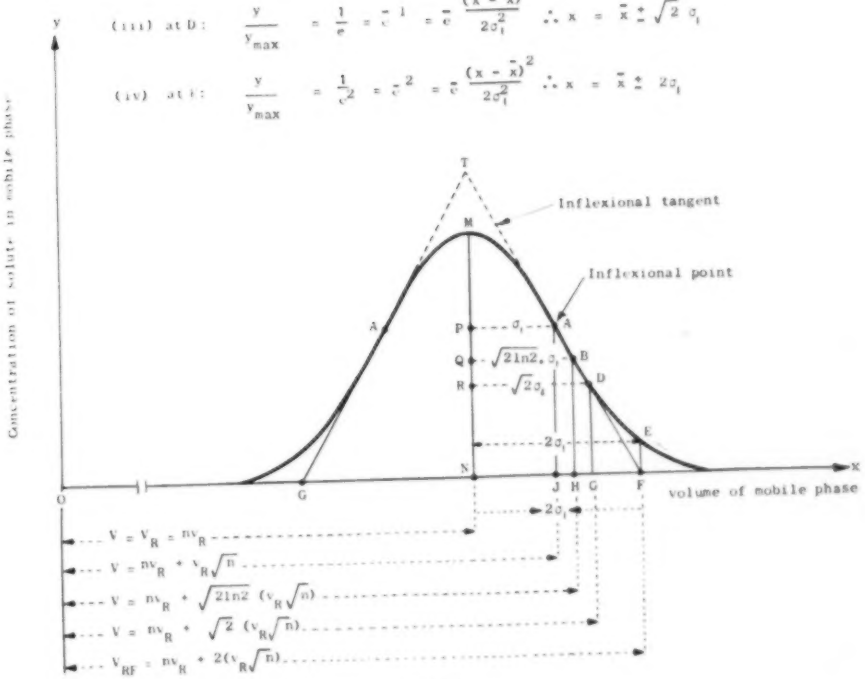


Fig. 7.

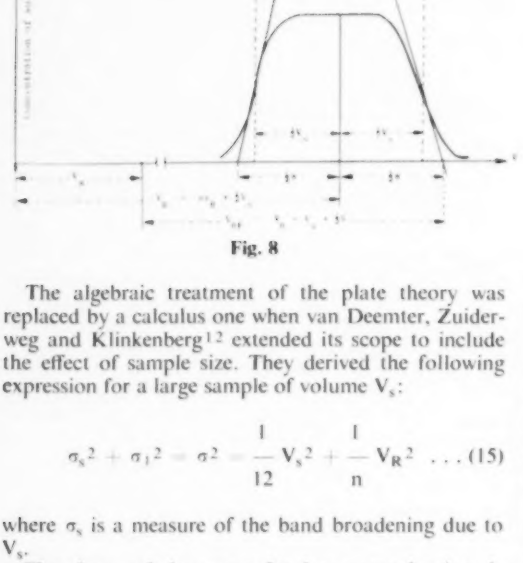


Fig. 8

The algebraic treatment of the plate theory was replaced by a calculus one when van Deemter, Zuiderweg and Klinkenberg[12] extended its scope to include the effect of sample size. They derived the following expression for a large sample of volume V_s:

$$\sigma_s^2 + \sigma_1^2 = \sigma^2 = \frac{1}{12} V_s^2 + \frac{1}{n} V_R^2 \quad \ldots (15)$$

where σ_s is a measure of the band broadening due to V_s.

The shape of the curve for large sample sizes is shown in Fig. 8.

It is obvious that when $\frac{1}{12} V_s^2$ is far less than $\frac{1}{n} V_R^2$, equation (15) reduces to equation (14) derived by Martin and Synge. Under this condition the flat top elution curve in Fig. 8 becomes a simple Gaussian curve. In fact van Deemter, Zuiderweg, and Klinkenberg (*loc. cit.*) showed that for practical purposes the shape of the elution curve is Gaussian and there would be no additional band broadening due to finite sample size if

$$V_s \leq \frac{1}{2\sqrt{n}} V_R = \frac{1}{2}\sqrt{n}\,\frac{V_R}{n} = \frac{1}{2}\sqrt{n}\,x$$

(retention volume per plate). . . (15a)

So long as the sample occupies less than $0{\cdot}5\sqrt{n}$ theoretical plates, there will be no band broadening due to sample size. Relation (15a) fixes the maximum space for the sample. Table I may help to explain the point.

It is clear from Table I that as the total number of theoretical plates in a column increases the maximum space, *in terms of theoretical plates*, to be occupied by the sample (which would no longer produce additional peak broadening effect) also increases. But the *percentage column length* available for the sample decreases with the increase in the number of theoretical plates.

TABLE I

No. of plates in the column	*Maximum space permissible for sample*	
	in terms of theoretical plates: $0{\cdot}5\sqrt{n}$	*in terms of % column length*: $\frac{0{\cdot}5\sqrt{n}}{n} \times 100$
4	1	25
100	5	5
400	10	2·5
10,000	50	0·5

(b) Rate Theory

The HETP is a useful concept, but it is an empirical factor; the plate theory does not directly explain the nature of the mechanisms which determine this factor. In order to gain insight of the different mechanisms governing the HETP, we have to make use of the rate theory. This theory is based on the use of parameters such as the rate of mass transfer between the two phases, the rate of diffusion along the column, and the rate of flow of the moving phase plus all other factors associated with the hydrodynamical behaviour of fluid flow.

Glueckauf[13] studied from a semi-kinetic angle the effect of four disturbing factors on chromatographic bands: (i) finite size of particle, (ii) longitudinal diffusion in the mobile phase, (iii) diffusion into the particle, (iv) diffusion in the mobile phase normal to the direction of flow. The theory was originally designed to describe the behaviour of an ion exchange column but its scope can be extended to other types of columns.

Van Deemter, Zuiderweg, and Klinkenberg gave a theory of the chromatographic process which in some ways is an alternative to the approach made by Glueckauf. These authors present a fairly full account of the various causes responsible for band broadening. The theory has been successfully applied to gas-liquid chromatographic columns which will be the subject of our subsequent discussion. Considering resistance to mass transfer between the two phases as arising from diffusion, they derived the following expression for HETP:

$$H = \underbrace{\frac{2D_e}{u}}_{\text{Contribution due to overall longitudinal diffusion}} + \underbrace{\frac{8}{\pi^2}\,\frac{k'}{(1+k')^2}\,\frac{d_f^2}{D_1}\,u}_{\text{Contribution due to resistance to mass transfer in the liquid phase}} \quad \ldots (16)$$

where

D_e is the effective or overall longitudinal diffusivity of solute in the gas phase,

$k' = K\frac{F_l}{F_g}$ is the capacity ratio (F_g is volume fraction of the gas and F_l that of the liquid phase),

K is the dimensionless partition coefficient defined in the usual way:

$$= \frac{\text{moles of solute in one cc. of liquid}}{\text{moles of solute in one cc. of gas}}$$

d_f is the 'effective depth' or 'effective film thickness' of the stationary liquid phase.

D_l is the diffusivity of solute in the liquid phase,

u is the apparent linear flow rate of gas phase.

Assuming that the overall longitudinal diffusivity (D_e) can be expressed as the sum of the longitudinal apparent diffusivity (D_a) and longitudinal (true) molecular diffusivity (D_g), we may write:

$$D_e = D_a + \gamma D_g \qquad \ldots (16a)$$

where the factor γ is empirically introduced to account for the irregular diffusion pattern due to the presence of solid particles. Since the molecular diffusivity in a packed bed is smaller than the free gas diffusivity in an open tube, γ is expected to be somewhat smaller than unity.

Klinkenberg and Sjenitzer[14] showed on statistical grounds that:

$$D_a = \lambda u d_p \qquad \ldots (16b)^*$$

where λ is a dimensionless constant characteristic of the mode of packing.

The factor λ is indicative of how good or bad the packing is. For regular packing λ is less than unity. For non-uniformly packed columns containing channels, λ is much higher than unity. It is easy to visualize how the band undergoes broadening or dispersion in a carelessly packed column streaked with channels. Molecules entering a given cross-section of the column simultaneously would pass through different path lengths and therefore spend different times to travel the same length of the column. In other words, some molecules get ahead while others lag behind the main band causing thereby an enhancement of the band width.

Band dispersion may also take place due to the uneven distribution of the stationary liquid on solid particles. Molecules entering the thinner part of the liquid film would permeate more quickly than the ones entering the thicker part. As a result of this, some molecules spend less time within the liquid phase and move faster whereas others spend more time and move more slowly than the rest of the band. So the band becomes wider.

Combining (16), (16a) and (16b), we have:

$$H = 2\lambda d_p + 2\gamma\frac{D_g}{u} + \frac{8}{\pi^2}\frac{k'}{(1+k')^2}\frac{d_f^2}{D_l}u \qquad \ldots (17)$$

It is clear from this equation that HETP is made up of three terms, the first one is independent while the other two are dependent on linear flow rate. To obtain high column efficiencies, it is essential to keep the magnitude of H as low as possible. For this purpose, we shall first study how to minimize the contribution of each term keeping the linear flow rate constant.

(i) The term $2\lambda d_p$ can obviously be reduced by reducing the size (d_p) of the particle. In practice, however, there is a limit to the extent to which d_p can be reduced. Experiments show that λ begins to increase as d_p is decreased showing thereby that it is easy to realize regular packing with large rather than with small particles. Experiments with 6 mm. diameter columns demonstrate that the most efficient packing is obtained within the limits of 30-80 mesh, the narrower the range the better. An additional advantage gained by using relatively large particles is the high permeability offered to the gas flow; thus high optimum gas velocities may be obtained without producing high pressure gradients which have adverse effect on column efficiency.

(ii) The term $\frac{2D_g}{u}$ can be reduced at constant flow rate by reducing D_g. From the kinetic theory of gases, the inter-diffusivity (D_g) of a given vapour in a gas depends upon the nature of the gas, the pressure and temperature of the system.* The rate of diffusion in low molecular weight gases such as H_2 or He is higher than that in high molecular weight gases e.g. N_2 or CO_2. Judging entirely on the basis of diffusion, it would seem that N_2 or CO_2 should be more beneficial in reducing HETP. However, looking from the angle of column operation, the choice of the mobile phase rests on several other factors. We shall have the opportunity of discussing this point at a later stage.

(iii) The expression $\frac{8}{\pi^2}\frac{k'}{(1+k')^2}\frac{d_f^2}{D_l}$ contributes to the HETP owing to slow diffusion of solute molecules in the stationary liquid phase. It may be

*The quantity D_a occurring in the above equations plays the same rôle in peak broadening as a diffusion coefficient. Also, if u is in cm. per sec. and d_p in cm.; then $2\lambda u d_p$ has the dimensions cm.^{2} sec.$^{-1}$. This represents the dimensions of a diffusion coefficient, hence the name apparent diffusion coefficient.

*The value of D_g as derived from the simple kinetic theory is given by:

$$D_g = \frac{0{\cdot}0043\ T^{\frac{3}{2}}}{P(V_b^{\frac{1}{3}} + v_b^{\frac{1}{3}})^2}\sqrt{\frac{1}{M}+\frac{1}{m}}$$, where M and m are the molecular weights and V_b and v_b the molar volumes at the normal boiling points of the solute vapour and the gas respectively.

remarked at the outset that owing to the interdependency of the various parameters involved in this expression, it is not possible to predict in general terms the variations in the magnitude of this expression. An immediately obvious method of reducing the magnitude of this expression is to reduce d_f which appears in the second power. This can be done by using smaller amounts of the stationary liquid; this means a reduction in F_l and hence a reduction in k' ($\frac{F_l}{F_g} K$). But a reduction in k' makes $\frac{k'}{(1+k')^2}$ bigger. So a decrease in d_f brings about an increase in $\frac{k'}{(1+k')^2}$. Also, any decrease in F_l is expected to produce an increase in F_g, since for a given column $(F_l + F_g)$ is constant. The manner in which $\frac{F_l}{F_g}$ or k' varies with d_f, or ultimately $\frac{k'}{(1+k')^2}$ varies with d_f^2, is not known exactly. However, experiments only indicate that columns impregnated with small quantities of stationary liquid are more efficient. If extremely lean columns are used, interference due to the adsorption of solute vapour on the naked surface of the solid particles sets in; this gives rise to tailing which impairs column efficiency. We also note that the capacity ratio k' ($K\frac{F_l}{F_g}$) depends upon the partition coefficient which in turn is a function of the solute-solvent interactions and the temperature. For the given solute and solvent, the partition coefficient generally increases with the decrease in temperature*. Assuming $\frac{F_l}{F_g}$ constant, we can increase K or k' and hence decrease $\frac{k'}{(1+k')^2}$ by decreasing the temperature. However, a decrease in temperature at the same time increases the viscosity† of the solvent decreasing thereby D_l*. Therefore the factors $\frac{k'}{(1+k')^2}$ and $\frac{1}{D_l}$ counteract each other, and it is not possible to tell exactly which of the two predominates and how the HETP is finally affected. The overall effect, of course, depends upon the system in hand and it is understandable why an increase in temperature some times produces a favourable effect while at other times an adverse effect on column efficiency.

It is clear from the above discussion that HETP is not only a function of the column packing, but it also depends upon the operating conditions and the nature of the solute. Under the same set of conditions, therefore, different values of HETP or different numbers of theoretical plates per unit column length may be expected for different solutes.

In the derivation of equation (17), it has been assumed that the resistance to mass-transfer lies almost entirely within the liquid phase; no account has been taken of the resistance to mass-transfer in the mobile phase. The absence of mass-transfer resistance in the mobile phase implies that there is complete mixing in this phase and concentration gradients normal to the net hydrodynamic flow (the resultant flow of the mobile phase through interstices) are non-existent. However, recent investigations show that there is in fact a resistance to mass-transfer in the gas phase and it can play a substantial part in controlling the HETP.

(To be continued.)

*The approximate thermodynamic relation between temperature and partition coefficient is:

$$\frac{d\ln K}{dT} = \frac{RT + \Delta H_s}{RT^2} \approx \frac{RT + \Delta\overline{H}_m - \Delta H_v}{RT^2}$$

where $H\Delta_s$ is the sum of heat of mixing $\Delta\overline{H}_m$ and the heat of condensation (– heat of vaporization = $-\Delta H_v$). The partition coefficient will decrease on increase of temperature for those systems which show a decrease in solubility and expand on absorption of heat.

†The influence of temperature on the viscosity of liquids is strikingly different from that of gases, the viscosity of liquids decreases appreciably as the temperature increases, whereas the viscosity of gases increases with the increase in temperature.

*If N_O be the Avogadro number, R the gas constant, η the viscosity of the liquid, and if the solute molecule is assumed to be a sphere of radius r_O, then according to the Stokes-Einstein diffusion equation:

$$D_l = \frac{R}{6\pi r_O N_O} \frac{T}{\eta};$$

the interdiffusivity (D_l) of the solute in the liquid, therefore, decreases as the temperature goes down.

REFERENCES

10Peters, W. A. (1922). Ind. Eng. Chem., **14**, 476.
11Martin, A. J. P. and Synge, R. L. M. (1941). Biochem. J., 35, 1359.
12van Deemter, J. J. Zuiderweg, F. J. and Klinkenberg, A. (1956). Chem. Eng. Sci., 5, 271.
13Glueckauf, E. (1949). Disc. Faraday Soc. No. 7, pp. 199-213.
14Klinkenberg, A. and Sjenitzer, F. (1956). Chem. Eng. Sci., 5, 258.

High-Voltage Paper Electrophoresis

The following corrections should be noted to the lecture 'High-voltage Paper Electrophoresis' by Dr. D. Gross published in the September issue of *Laboratory Practice*, pages 622 *et seq.*:

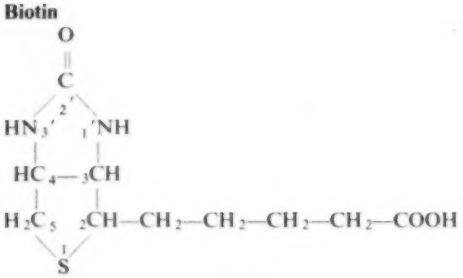
p. 623, column 1, 11th line from the bottom:
temperature to read time.
ibid., 5th line from the bottom:
insert > to read for > 50 V./cm.
p. 627, column 1, 6th line from the bottom:
insert > to read of > 50 V./cm.

PRACTICAL METHODS FOR THE MICROBIOLOGICAL ASSAY OF THE VITAMIN B-COMPLEX AND AMINO ACIDS

by E. C. Barton-Wright, D.Sc., F.R.I.C., M.I.Biol.

PART III

Biotin



Biotin
(2′ keto-3:4-imidazolido-2-tetrahydrothiophene-*n*-valeric acid)

Organism. Lactobacillus plantarum.

Basal Medium

With some slight modifications, the same basal medium as that used for the assay of nicotinic acid may be employed for the assay of biotin. The basal medium described here is essentially the same as that of Lynes and Norris (1948) with a few minor modifications introduced by the author. The composition of the medium is as follows:

Vitamin-free casein hydrolysate 1·0 per cent, L-cystine 0·01 per cent, DL-tryptophan 0·02 per cent, glucose 1·0 per cent, sodium acetate 0·9 per cent, adenine, guanine, uracil and xanthine 10 p.p.m. each, thiamine 1·0 p.p.m., riboflavin 0·5 p.p.m., pyridoxine 1·0 p.p.m. calcium *d*-pantothenate 1·0 p.p.m., nicotinic acid 2·0 p.p.m., *p*-aminobenzoic acid 0·1 p.p.m., sodium chloride 0·5 per cent, ammonium sulphate 0·3 per cent, dipotassium hydrogen phosphate 0·06 per cent, potassium dihydrogen phosphate 0·06 per cent, and inorganic salt solution.

Biotin-free Basal Medium
(Sufficient for 100 tubes)

Vitamin-free casein hydrolysate*	10·0 g.
L-Cystine solution*	25·0 ml.
DL-Tryptophan solution*	50·0 ml.
Glucose	10·0 g.
Sodium acetate (hydrated)	15·0 g.
Adenine + guanine + uracil solution* ...	10·0 ml.
Xanthine solution*	10·0 ml.
Sodium chloride	5·0 g.
Ammonium sulphate	3·0 g.
K_2HPO_4	0·6 g.
KH_2PO_4	0·6 g.
Thiamine*	1,000 μg.
Riboflavin*	500 μg.
Pyridoxine*	1,000 μg.
Ca-*d*-pantothenate*	1,000 μg.
Nicotinic acid*	2,000 μg.
p-Aminobenzoic acid*	100 μg.
Inorganic salt solution*	5·0 ml.
Water to	500 ml.

*For the preparation of these reagents see Part III.

After mixing, the pH of the medium is adjusted to 6·8 with sodium hydroxide solution using bromothymol blue as external indicator, and the volume made up to 500 ml. with water.

Assay Procedure

Assay procedure is exactly the same as for nicotinic and pantothenic acids. The basal medium is at double strength and 5 ml. lots are transferred to test tubes; the standard biotin solution and test solutions are added in the usual way and the total volume of liquid in each tube made up to 10 ml. with water.

The effective range for establishing a standard curve is: *Blank*, 0·00005 μg., 0·0001 μg., 0·00015 μg., 0·0002 μg., 0·00025 μg., and 0.0005 μg. A standard solution of biotin is prepared to contain 0.0001 μg./ml. and the rest of the operation is carried out as for nicotinic and pantothenic acids. The assay of test extracts is carried out at three or four different concentration levels and all concentration levels are set up in duplicate if response is to be measured acidimetrically or in triplicate if response is to be determined nephelometrically. After the volume in each tube has been adjusted to 10 ml. with water, the tubes are capped with aluminium thimbles and sterilized as for nicotinic and pantothenic acids. The temperature of incubation if response is to be determined nephelometrically is 35-37° C. and 30° C. if response is to be measured acidimetrically. The inoculum is prepared in the same way as for nicotinic and pantothenic acids.

Preparation of Sample for Assay

The finely ground sample is hydrolysed with 3 N. sulphuric acid at 15 p.s.i. for 30 minutes, cooled, 2 ml. of 2·5 M. sodium acetate solution added and the pH. adjusted to 4·5 with sodium hydroxide solution using bromocresol green as external indicator, made up to volume and filtered. The pH of a suitable aliquot is adjusted to 6·8 and made up to a suitable volume for assay. Materials with a high fat content, e.g. wheat germ, oats, meat, etc., should be subjected to a preliminary extraction with light petroleum.

Practical Hints in the Preparation of Ingredients for Media for Assay of Nicotinic Acid, Pantothenic Acid and Biotin

Distilled Water.—Glass distilled water has been recommended for the preparation of stock solutions, basal media and extracts. The author now uses a Manesty still and has found this to be perfectly satisfactory. Nevertheless, as Price and Gare (1959) (also a private communication from S. A. Price) have pointed out, most workers have occasionally encountered trouble with sub-optimal growth in some tubes and irregular replicates even with well-established routine assay procedures. Price and Gare were able to trace their own difficulties to the presence of a volatile inhibitor in their distilled water; possibly a short-chained fatty acid, which could be removed by 'degassing' the distilled water by boiling down to 50 per cent of its original volume.

Washing of Glassware.—Careless washing of glassware is a prolific cause of invalid assays and far too little attention has been paid to this important subject. A number of different methods have been suggested in the past, all of them more or less unsatisfactory. The following procedure will be found to give uniformly satisfactory results.

All glassware, test tubes, screw-capped bottles etc., are boiled in a 2-gallon stainless steel bucket for 10 minutes in the following solution:

Lux soap flakes	4·0 g.
Calgon	6·0 g.
Distilled water	8 litres

After boiling, the bucket is allowed to stand until the solution is warm to the hand. The glassware is then transferred to a basin or other suitable receptacle and rinsed three times in 8 litres of tap water plus 1 oz. of Calgon. The glassware is next rinsed three times in plain tap water and finally twice with distilled water, inverted and dried.

Toluene.—Toluene used for preserving stock solutions must be sulphur-free; alternatively chloroform may be used as a preservative.

Sodium Acetate.—Hydrated sodium acetate should always be used in preference to the anhydrous salt which is frequently contaminated with iron (1·0 g. anhydrous sodium acetate ≡ 1·66 g. of hydrate).

Vitamin-free Casein Hydrolysate.—A number of preparations of acid-hydrolysed vitamin-free casein suitable for microbiological assay have been placed on the market from time to time, but the product prepared by Difco Laboratories is strongly recommended for its consistently high quality, so that nowadays it is quite unnecessary for the individual worker to prepare his own material. Should the necessity arise for the preparation of this material in the laboratory, either of the methods described below will be found to give a satisfactory product.

Method 1. 120 g. of a 'vitamin-free' casein are shaken twice with 300 ml. of 85 per cent ethanol for one hour and filtered. The ethanol-washed casein is allowed to stand overnight in 500 ml. of 85 per cent ethanol, filtered, and washed with water at 50° C. The ethanol-extracted casein is now hydrolysed with 500 ml. of 25 per cent sulphuric acid at 15 p.s.i. for 10 hours in an autoclave, or refluxed on a sand bath for 16-20 hours. The hydrolysate is neutralized with either barium or calcium hydroxide, the pH adjusted to 3·0 with sulphuric acid and then shaken for 30 minutes with a good quality activated charcoal, such as Norite, filtered, and neutralized with barium or calcium hydroxide and the precipitated sulphate filtered off. The resulting hydrolysate should be water-clear. If calcium hydroxide is used for neutralization, excess calcium should be removed by adding the calculated quantity of oxalic acid. The solution is adjusted by evaporation or dilution to contain 100 mg./ml. of solid matter and stored under toluene or chloroform in a refrigerator. A precipitate of tyrosine is invariably thrown down after a few days, but this may be disregarded.

Method 2. After ethanol extraction in the manner described above, 120 g. of casein are hydrolysed by refluxing for 14-16 hours with 300 ml. of concentrated hydrochloric acid on a sand bath. **This operation must be carried out in a fume cupboard.** The hydrolysate is taken down under reduced pressure to remove excess hydrochloric acid, diluted with water and the process repeated. Litharge is now added with constant stirring until a pH of 3·0 is reached. The precipitated lead chloride is filtered off and hydrogen sulphide passed into the hydrolysate to precipitate any lead chloride left in solution. Excess hydrogen sulphide is removed by aeration and the mixture shaken with 20 g. of active charcoal for 30 minutes and filtered. The resulting hydrolysate should be water-clear. The solution is adjusted by dilution or evaporation to contain 100 mg./ml. of solid matter. There is no need to readjust the pH of the hydrolysate to 6·8. It is stored in a refrigerator under toluene or chloroform. Tyrosine invariably precipitates after a few days and may be ignored.

Sodium Hydroxide-treated Peptone.—40 g. of Difco peptone are dissolved in 250 ml. of water and 20 g. of sodium hydroxide, also dissolved in 250 ml. of water, added. The mixture is brought to the boil and *boiling should not be continued for more than two or three seconds.* The mixture is cooled under the tap and incubated for 48 hours at 37° C. At the end of this time, the solution is neutralized with glacial acetic acid (ca. 28 ml.) and 11·6 g. of sodium acetate added. The solution is made up to 800 ml. with water and stored in the refrigerator under toluene or chloroform. This solution will keep for two or three weeks, but should a precipitate appear, or should the solution become cloudy, it should be discarded.

L-Cystine Solution.—1·0 g. of L-cystine is suspended in 25-30 ml. of water and boiled. Concentrated hydrochloric acid is added dropwise until the whole of the cystine passes into solution. The volume is then made up to 250 ml. and the solution is stored under toluene in a refrigerator. This solution contains 4 mg./ml. of cystine and keeps more or less indefinitely.

DL-Tryptophan Solution.—1·0 g. of DL-tryptophan is boiled in 25-30 ml. of water and concentrated hydrochloric acid is added dropwise until solution is effected. The solution is made up to a final volume of 250 ml. and stored under toluene in a refrigerator. It keeps almost indefinitely. This solution contains 4 mg./ml. of DL-tryptophan.

Adenine, Guanine and Uracil Solution.—The stock solution of these three substances contains 1·0 mg./ml. of each. Solution is effected by boiling with a little concentrated hydrochloric acid. The solution is stored in a refrigerator under toluene and should be renewed at monthly intervals.

Xanthine Solution.—This solution also contains 1·0 mg./ml. Solution is effected by boiling with a few drops of concentrated ammonia. This solution is stored under toluene in a refrigerator and should be renewed at monthly intervals.

Thiamine Solution.—Thiamine (0·1 g.) is accurately weighed and dissolved in a little water and made up to a final volume of 100 ml. with 2 per cent hydrochloric acid. This solution contains 1,000 μg./ml. It will maintain its activity for several weeks if stored in a refrigerator.

Calcium d-*Pantothenate Solution.*—0·1 g. of calcium *d*-pantothenate is dissolved in water and the volume made up to 100 ml. The solution is stored under toluene in a refrigerator and renewed at fortnightly intervals. This solution contains 1,000 μg./ml.

Pyridoxine Solution.—Pyridoxine hydrochloride (0·244 g.) is dissolved in 100 ml. of water, and stored under toluene in a refrigerator. This solution contains 2,000 μg./ml. of pyridoxine (free base). The solution should be renewed at monthly intervals.

Nicotinic Acid Solution.—0·1 g. of nicotinic acid is dissolved in 100 ml. of water and stored under toluene in a refrigerator. This solution contains 1,000 μg./ml. It should be renewed at weekly intervals.

Riboflavin Solution.—50 mg. of riboflavin are accurately weighed out, mixed with a little water and 1·0 ml. of glacial acetic acid added, and the whole made up to 1,000 ml. with water. This solution contains 50 μg./ml. of riboflavin. The solution is stored under toluene in a refrigerator and renewed at monthly intervals.

p-*Aminobenzoic acid.*—0·1 g. of *p*-aminobenzoic acid is dissolved in 100 ml. of water with the aid of a little glacial acetic acid. This solution contains 1,000 μg./ml. It is stored under toluene in a refrigerator and renewed at monthly intervals.

Biotin Solution.—50 mg. of biotin are accurately weighed out and dissolved in a little water and made up to a final volume of 1,000 ml. with water and 200 ml. of ethanol. This solution contains 50 μg./ml. of biotin and will keep in a refrigerator for three months without deterioration.

Inorganic Salt Solution. (Formerly Inorganic Salt Solution B). 10 g. of $MgSO_4$, $7H_2O$, 0·5 g. $MnSO_4$, $4H_2O$ and 0·1 g. of $FeCl_3$ (anhydrous) are dissolved in 250 ml. of water and a few drops of concentrated hydrochloric acid added to prevent precipitation. This solution will keep indefinitely at room temperature.

Assessment of Validity of Microbiological Assays and Methods of Computation

Typical standard curves for the assay of nicotinic acid, pantothenic acid and biotin are shown in Figs. 1, 2 and 3. It will be seen that the standard curve for nicotinic acid contains a strictly linear portion, whereas the curves for biotin and pantothenic acid are curvilinear throughout their length. The method of computation which will have to be adopted will necessarily depend upon the nature of the standard curve obtained for a particular assay. If there is a linear portion (nicotinic acid) to the standard curve, then the slope-ratio method should be employed, on the other hand, if the curve is curvilinear (pantothenic acid and biotin) the 'log-log' method devised by Wood (1945) should be used. Examples of how to use these two methods of computation are given below.

The successive steps for calculating an assay result by the slope-ratio method are as follows:

(1) The mean responses to the standard solutions (either the mean titration figure in ml. of standard solution, or the mean nephelometric figure) are plotted on a piece of squared paper against the doses in μg. The response of the zero-dose or blank should be

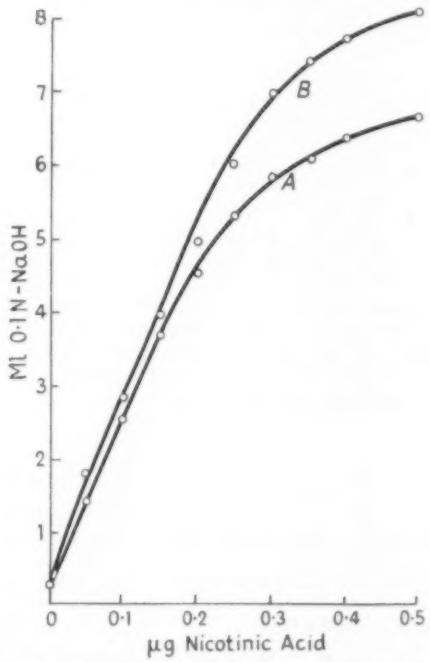


Fig. 1. A—**standard curve obtained on original Snell and Wright medium.** B—**standard curve obtained on modified medium.**

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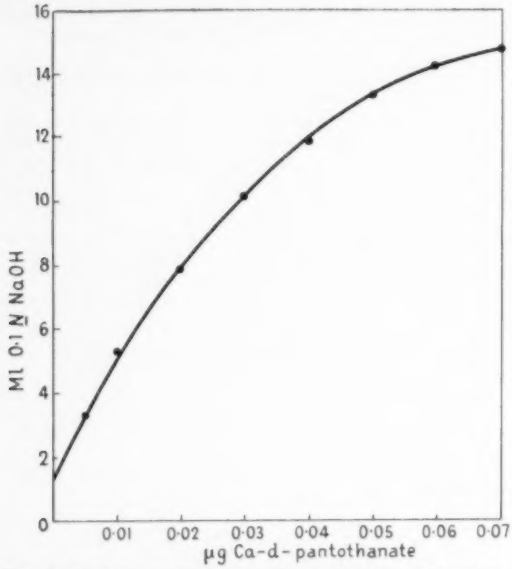

Fig. 2. Typical standard curve for pantothenic acid using *Lactobacillus plantarum.*

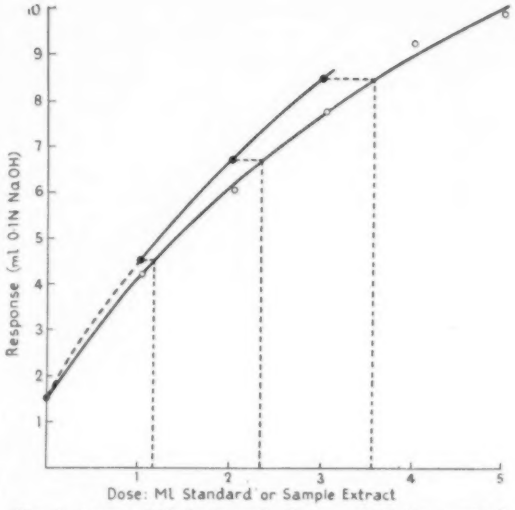

Fig. 3. Assay of biotin in malt. O———O biotin standard. ●———● sample extract.

included. The best fitting straight line is then drawn between the plotted points. If the point corresponding to the blank or zero-dose does not lie on the 'best' line through the points it should be ignored.

(2) The points of the various doses of the test preparations are plotted in the same way on the same graph. The scale used is immaterial, so long as the zero corresponds with that of the standard solution graph. The 'best' line is now drawn through these points.

(3) The next step is to calculate the slopes for each line in turn. From any two convenient points on the graph the values for dose and response are read off at opposite ends of the line. The lower dose is now subtracted from the higher dose and the lower response from the higher response in order to obtain the increase in response for a given increase in dose. From the slope is calculated the increase in response per unit of standard solution or test solution as the case may be.

(4) Whether or not an assay is valid may be detected at once by this method of calculation. Ideally the two lines will intersect exactly on the vertical axis of response in a valid assay; a slight divergence may be disregarded.

Example.—The dose levels used and the mean titres obtained in an assay of a meat extract for nicotinic acid are given in Table 4.

It will be seen from Fig. 4 that the standard points, including the blank, all lie very close to a straight line and the same applies to the three test points, which also lie on another straight line. It should also be noted that the two lines almost intersect on the vertical axis of response.

The actual calculation is carried out as follows: the values of response, read off by eye, from the standard line, which passes from zero-dose to 0·25 µg. of nicotinic acid, are respectively 1·60 ml. and 9·43 ml., which gives an increment of 7·83 ml. for 0·25 µg. of

TABLE IV

Assay of Nicotinic Acid

(Data from Kent-Jones and Meiklejohn, 1944)

Dose of Nicotinic acid (µg.) ...	0·0	0·05	0·10	0·15	0·20	0·25
Mean titration (ml.)	1.45	3.35	4.85	6.15	7.85	9.45
Weight of test extract (mg.) ...	0·20		0·30		0·40	
Mean titration (ml.)	4·85		6.40		7·70	

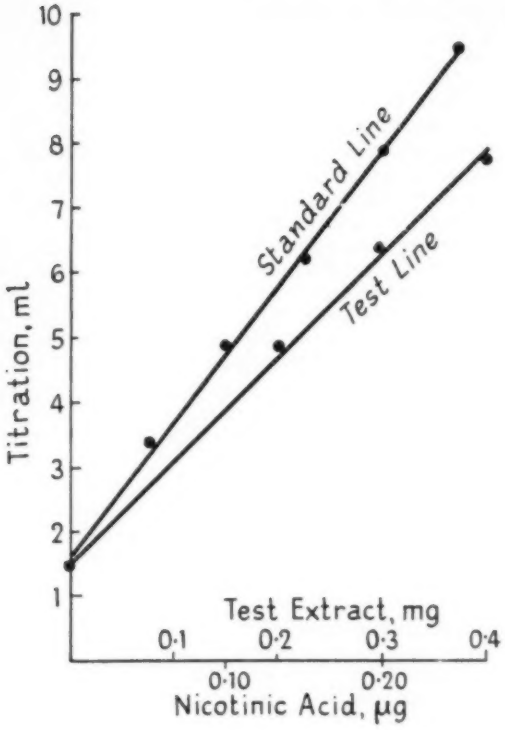

Fig. 4.

nicotinic acid, or a slope of 7·83 × 4 = 31·32 ml./µg. The values of response from the test line are treated in exactly the same way. The values here, corresponding to zero-dose and 0·40 mg. of meat extract are 1·55 ml. and 7·85 ml. respectively, giving an increment of 6·3 ml. for 0·40 mg., or a slope of 6·3 × 2,500 = 15,750 ml./g. The mean nicotinic acid content of the meat extract is therefore given by the ratio of the two slopes, i.e. 15,750/31·32 = 503 µg./g.

This result may be taken as a good example of a valid assay, because not only do all the points lie quite close to the appropriate line, but the distance between the points of intersection of the standard line with the response axis and the test line with the same axis is negligible (0·05 ml.).

The slope-ratio method of computation for microbiological assays has unfortunately very limited use. It can only be applied to four assays: nicotinic acid, riboflavin assayed with *L. helveticus*, thiamine assayed with *L. fermenti* 36 and cyanocobalamin assayed with *Ochromonas malhamensis*, and not always with these. In all other microbiological assays the standard curves are curvilinear throughout their length. For a number of years the only method of computation available with standard curves of this nature was to read off the values

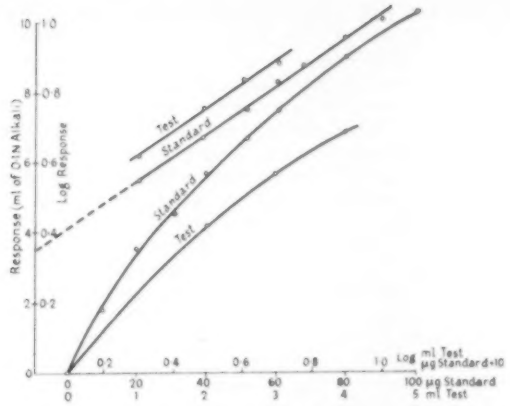

Fig. 5. Assay of threonine with *Streptococcus faecalis*.

at different concentration levels of the test solution from the standard curve, which was a very unsatisfactory procedure. Any transformation that could be effected for such curves, so that they would give linear graphs would be a great advantage, because it would supply a sound mathematical basis of computation. Wood (1945) was able to effect such a transformation by plotting the logarithms of both the doses and responses. The resulting points were found to lie reasonably well on a straight line over a certain range.

A good example of an assay in which this 'log-log' relationship holds is shown in Fig. 5 which is an assay of threonine using *Streptococcus faecalis* R. It will be observed that the curves obtained by plotting dose against response, both for the standard curve and for the test preparation are transformed into two parallel straight lines when log-dose is plotted against log-response. In a valid assay the test line must lie parallel to the standard line. Any significant lack of parallelism would indicate that the assay is invalid.

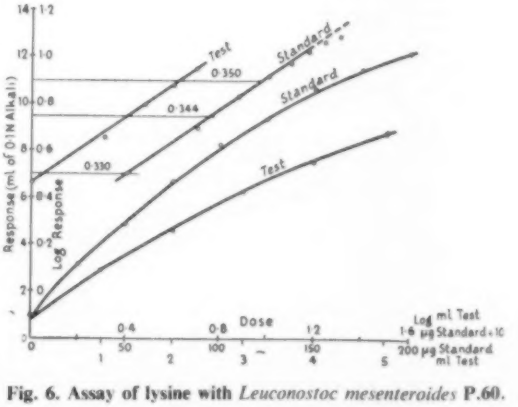

Fig. 6. Assay of lysine with *Leuconostoc mesenteroides* P.60.

The parallelism shown by the standard line and test line in Fig. 5 is of a very high order, but in an assay of lysine using *Leuconostoc mesenteroides* P.60 (Fig. 6) it will be seen that parallelism, although good, is not quite so exact as in the threonine assay.

The best and quickest method of computing the result of an assay after the 'log-log' transformation has been effected is to measure the horizontal distance from test line to standard line. Thus, taking any point on the log-response axis, draw a parallel line to the log-dose axis (Fig. 6) and read off the values for log-dose corresponding to the points of intersection made by this line and the test and standard lines. In this case, the horizontal distance between the standard and test lines along the line meeting the log-response axis at 0·75 is 0·76 − 0·421 = + 0·344. This figure represents log (ml. standard solution equivalent to 1 ml. test extract), on adding the value for the logarithm of the potency of the standard solution (μg./ml.) this figure then represents log (μg. vitamin or amino acid equivalent to 1 ml. test extract). On taking the antilogarithm and knowing the concentration of the test extract, the vitamin or amino acid content of the test material is readily ascertained.

In this assay the standard solution was pure lysine and the unit 10 μg. and the logarithm therefore 1·000. From this we obtain that 1 unit of the test extract which in this case is 1 ml. contains antilogarithm 1·344 = 22·1 μg. lysine. But in this particular assay, test and standard lines are not quite parallel, and in such circumstances it is best to make a measurement near the midpoint of the two lines. A further test of validity of an assay by this method is to make the measurements near the extreme limits of linearity and examine the agreement between the two resulting estimates of potencies. Should agreement be good, the mean may be taken as the answer. In this assay at the log-response of 0·5 and 0·9, the corresponding estimates of potency of the test extract are 21·5 and 22·5 μg./ml. with a mean of 22·0 μg. Thus, the extremes here are within 3 per cent of the mean which is a satisfactory result.

REFERENCES

Lynes, K. J. and Norris, F. W. (1948). J. Inst. Brew., **45**, 150.
Kent-Jones, D. W. and Meiklejohn, M. (1944). Analyst, **69**, 330.
Price, S. A. and Gare, L. (1959). Nature, **183**, 838.
Wood, E. C. (1947). Analyst, **72**, 84.
Wood, E. C. (1945). Nature, **155**, 632; (1946). Analyst, **71**, 81.

Additional References

Wood, E. C. and Finney, D. J. (1946). Quart. J. Pharm. Pharmacol. **19**, 112.
Wood, E. C. (1947). Chem. Ind., 334.

(To be continued)

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Correct as at June 1, 1961

THERMOGRAVIMETRIC ANALYSIS

by G. M. Lukaszewski, Ph.D., and J. P. Redfern, Ph.D., A.R.I.C.

Battersea College of Technology

PART IV—SOME THEORETICAL CONSIDERATIONS

THE purpose of this article is to give a brief discussion of some of the theoretical aspects of the technique of thermogravimetric analysis. Its applications have been considered in relation to a wide variety of examples from different branches of chemistry. Some of the qualitative and semi-quantitative interpretations which are relatively simple have already been included in the discussion, but the more detailed interpretation involves a study of solid-solid transformations.

Kinetics and Reactions in Solid-solid Transformations

Certain essential differences exist between reactions in the solid phase and reactions occurring either in the gas phase or in solution. In the last two phases the reactant molecules, which are in motion, become available for reaction with one another under conditions defined by relatively simple statistical laws. In contrast, solid state reactions occur between molecules in apparently regular lattices in which motion is restricted, and thus interaction can only occur at points of intimate contact between the reacting phases. In gas or solution reactions the governing factor is a 'mass action' effect, whereas in solid state reactions a relatively immobile product layer builds up at the initial point of contact between the reacting species so that continued reaction will depend on some diffusion process by which reactants can pass through the product layer. Thus the principal factor governing the rate of a solid-solid transformation will be a rate of particle transfer through the product layer and the rate of the boundary process occurring between the initial compound and the newly-formed phase.

The nature of solid-solid reactions is very complex, and although it is generally assumed that simple stepwise processes of decomposition are involved, it is quite often found that the reactions proceed through a series of intermediate stages. These stages may involve the concomitant formation and subsequent decomposition of several solid phases. It is then that thermogravimetric analysis and the associated studies previously described can be used to provide information regarding the reaction mechanism.

Solid-solid transformations may be classified under the following headings:

(i) Reactions involving a decomposition such as:
$A_{(solid)} \longrightarrow B_{(solid)} + C_{(gas)}$

(ii) Reaction between two solid phases:
$A_{(s)} + B_{(s)} \longrightarrow AB_{(s)} + C_{(g)}$

(iii) Reactions confined exclusively to the solid phase:
e.g. $A_{(s)} + B_{(s)} \longrightarrow C_{(s)} + D_{(s)}$

(iv) Reactions involving participation of liquid phases at some stage.

(v) Reactions involving a solid and a gas (i.e. from a controlled atmosphere or oxygen from the atmosphere):
$A_{(s)} + B_{(g)} \longrightarrow C_{(s)}$

Such reactions can often occur consecutively or simultaneously. In general, thermogravimetric analysis is concerned with single substances and the two main initial reactions are thus (i) and (v). Thermally initiated reactions which involve the entry or substitution of an anionic ligand into the co-ordination sphere of an inorganic complex compound, result in the loss of a neutral ligand initially present (e.g. H_2O, NH_3, or other volatiles) may involve reactions of type (i) and (ii).

Thus

$$[M(H_2O)_n]\,X_{(s)} \longrightarrow [M(H_2O)_{n-1}\,X]_{(s)} + H_2O_{(g)}$$

and

$$[M(H_2O)_{n-1}\,X]_{(s)} + [M(H_2O)]_nX_{(s)} \longrightarrow$$

$$[(H_2O)_{n-1}\,M\begin{matrix}X\\ \diagdown\\ \diagup\\ X\end{matrix}M\,(H_2O)_{n-1}]_{(s)} + H_2O_{(g)}$$

This type of process is of considerable importance in inorganic chemistry.

The first stage in an experimental study of a thermal decomposition would be to obtain the pyrolysis curve using the thermobalance. For a single stage reaction curves of the type shown in Fig. 14 are obtained where weight loss is plotted against time while the pressure is maintained constant. In general their shape is found to

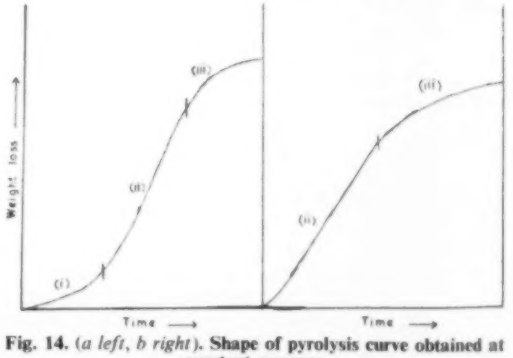

Fig. 14. (*a left, b right*). **Shape of pyrolysis curve obtained at constant pressure.**

be sigmoid, which is indicative of an autocatalytic reaction. The curve in Fig. 14a may be divided into three parts: (i) an induction or accelerating period, (ii) main portion of the curve where the fractional weight loss is proportional to the time, (iii) a final decay period. In some cases the induction period is of short duration or virtually absent while the final decay period is more pronounced, resulting in a somewhat asymmetrical curve (Fig. 14b). Garner (1955) has reported that factors such as grinding, crushing, or scratching the surface of crystalline materials will reduce the induction periods; the method of preparation may also affect the thermal stability of the substance.

The majority of reactions studied have been of type (i) where the onset of the reaction involves the formation of a new phase B at certain points of the lattice of A. Decomposition will commence where local fluctuations provide favourable circumstances for the formation of B. Further reaction will tend (due to B's presence and catalyzing effect) to take place at the boundary between the solid phases. The product phase B will spread out from the points where the reaction initially commenced. Such points are referred to as 'nuclei'. Generally the induction period is related to the initiation of nuclei formation in the reactant, which may be brought about thermally, in which case the induction period will be apparent in the decomposition graph; or physically, e.g. by scratching, grinding, etc., when the induction period is reduced. The general form of decomposition curves can thus be explained qualitatively in terms of nucleus formation and growth. Thermogravimetric work involves a continuous linear rise in temperature, and the shape of the decomposition curve is modified accordingly, so that the main portion of the curve will be considerably steeper than it would be were the temperature kept constant. It is important to recognize that the third phase of a decomposition may overlap with the induction period of a new reaction. This may result in unsatisfactory resolution of consecutive stages. Thus the quasi-static approach to a problem is useful, after initial information relating to a series of decompositions has been obtained using a linear temperature-rise programme.

The discussion applies equally to both endo- and exothermic reactions. The former are usually processes of type (i) or (ii), whilst the latter embrace all types but more particularly those which involve major structural alterations.

The majority of studies on endothermic reactions have been confined to the decomposition of hydrates and carbonates. With hydrates the residues on dehydration consist either of a lower hydrate or an anhydrous salt. Three types of behaviour have been recognized:

(i) where the crystal lattice of the residue is practically identical with that of the constituents of the original hydrate.

(ii) where a phase showing little crystalline structure is produced.

(iii) where the dehydrated residue crystallizes to give a new solid phase.

With inorganic compounds (i) only occurs with simple hydrates, (ii) commonly occurs with hydrates of multivalent elements, especially at higher temperatures, and (iii) occurs principally in pyrolysis. Thus Shafer and Roy (1956) and Sullivan and McMurdie (1952) have studied chromium orthophosphate, which between the temperatures of 300° and 950° C shows behaviour of type (ii), whilst at around 1,300° new crystalline phases, designated α and β forms, of type (iii) are produced. These workers used x-ray diffraction studies to provide confirmatory evidence of the transition from the amorphous to the crystalline form. In many hydrates a process of elimination may be occurring with the production of polymeric materials. Such processes are generally referred to as *olation reactions* (see e.g. Rollinson, 1956) and result in the formation of hydroxy or oxo bridging. Similar behaviour may be observed with certain anions and may be termed *oxyanionolation*. The use of thermogravimetric techniques coupled with the use of magnetic and infra-red studies provide a powerful method for the elaboration of such processes (Lukaszewski, Redfern and Salmon, 1960). The full removal of water in the course of dehydration is important because this may markedly alter the kinetics of the process due to a catalytic effect. Hepburn and Phillips (1952) obtained better results with hydrate transitions carried out *in vacuo* but the adoption of this technique to thermogravimetric study is not easy.

The rate of dehydration is determined by two factors:

(i) The rate of water loss from the interface of the hydrate and its products.

(ii) The rate of diffusion of the water molecules through the solid product. Topley (1932) have shown that the 'impedance' to the escape of water has a considerable effect on the rate of the process.

Freeman and Carroll (1958) have discussed the application of thermoanalytical techniques to the investigation of rate processes. Equations were derived for irreversible reactions which may be used to calculate the energy of activation and the order of a reaction from thermogravimetric results. These workers have discussed the theoretical treatment of Borchardt and Daniels (1957) relating to the evaluation of these parameters from results obtained by D.T.A. and modified their treatment so as to eliminate the trial and error procedure. From thermogravimetric results Freeman and Carroll derived the equation

$$\frac{\dfrac{-E^*}{2{\cdot}3R}\,\Delta(1/T)}{\Delta \log W_r} = -x + \frac{\Delta \log \dfrac{dw}{dt}}{\Delta \log W_r} \qquad \ldots\ldots(1)$$

where E^* is the activation energy of the reaction, x is the order of the reaction, T the absolute temperature,

R the general gas constant, $W_t = w_\infty - w$ where w_∞ is the weight loss at the completion of the reaction and w the total weight loss up to time t. A plot of

$$\frac{\Delta \log dw/dt}{\Delta \log W_t} \text{ against } \frac{\Delta (1/T)}{\Delta \log W_t}$$

should result in a linear plot of slope $= \frac{E^*}{2{\cdot}3R}$ and an intercept corresponding to x. The advantages of evaluating these reaction parameters from thermogravimetric results obtained with a linear rise in temperature are that considerably less experimental data is required than in the case of an isothermal approach and the kinetics can be probed over an entire temperature range in a continuous manner without any interruptions. These workers have studied the thermal decomposition of calcium oxalate monohydrate by this method. Lumme (1959) has studied divalent metal complexes and applied the Freeman-Carroll calculations most successfully.

Very few detailed investigations have been reported for organic compounds in the solid state. The forces in organic crystals are largely of the Van der Waals type, although in certain cases considerable electrostatic forces may be involved. The intermolecular forces are small in comparison to the energy required for bond dissociation. As a consequence melting and sublimation occur at relatively low temperatures so that one or both of these takes place before actual thermal decomposition. Such changes imply a departure from solid state conditions and thus do not come within the scope of this discussion.

Essentially the decomposition of organic materials shows a number of characteristic differences from that of inorganic materials;

(i) It has never been established that initial decomposition occurs at 'nuclei' only.
(ii) Decomposition is often accompanied by the formation of a liquid phase and hence a rapid increase in the reaction rate is observed.
(iii) Decomposition generally leads to the formation of some gaseous products. In fact these reactions have received the most study.

It has been shown by Prout and Tompkins (1944) that the decomposition curves for organic materials are generally sigmoid in shape, although not for the same reasons as inorganic compounds. Interpretation has been based on a chain-reaction theory which accounts for the shape of the curve and the appearance of a well-defined induction period which has been observed in certain cases. The possibility of autocatalysis has been discussed by Garner (1955).

The decomposition of many organic and inorganic materials is accompanied by the liberation of considerable quantities of heat energy. If the rate of heat production is greater than the rate of heat loss to the surroundings (by convection and conduction) self-heating is likely to occur, and the reaction accelerates to a high velocity, often resulting in inflammation and explosion. Garner has referred to such behaviour as 'thermal explosion'.

Phase Rule Considerations

Early work in the field of dehydration studies was based on the classical phase rule. Such an approach was justified by the results obtained with such well-defined salt hydrates and hydroxides as $CuSO_4.5H_2O$ and $Ca(OH)_2$. These compounds gave sharp discontinuities at points in the temperature-composition diagrams, corresponding to the release of water vapour. However the inadequacy of the classical phase rule applied to solid-solid transformations has been stressed by Ubbelohde (1957). He pointed out that certain energy terms, due to the presence of internal surfaces, and strains characteristic of many solids were not allowed for in the classical derivation of the phase rule. Inclusion of such terms has the effect of increasing the number of degrees of freedom available to the system. Consequently the phase rule equation (2) should be modified as in equation (3).

$$F = C - P + 2 \quad \ldots\ldots\ldots\ldots (2)$$

$$F = C - P + 2 + \Sigma\pi \quad \ldots\ldots\ldots (3)$$

where $\Sigma\pi$ refers to the additional degrees of freedom introduced. Transformations which behave in accordance with classical thermodynamics are termed 'discontinuous' and those which show no discontinuity are referred to as 'continuous', i.e. where there is evidence that 'continuity' arises through a coexistence of two distinct structures over a narrow range of temperature in a hybrid single crystal. Such continuous transformations are best described as smeared transformations from one structure to another.

Although the phase rule predicts sharp decompositions at specific temperatures and pressures, the thermal dehydration curves for many compounds are frequently sigmoid in shape. Rounding of the curve in the early stages of dehydration (the 'forewarning' of a solid-solid transformation) had been attributed to the influence of varying particle size upon the equilibrium vapour pressure of the solid, but Garner (1955) has argued that the phenomenon can best be attributed to kinetic factors such as 'spontaneous nucleation'. It has also been suggested that the rounding of the terminal portion of the curve might be due to the retardation of water by strong adsorption on the dehydration product. However, although it seems plausible to attribute small terminal losses to this cause it is probably not the main mechanism involved. Whatever the mechanism causing this type of behaviour its existence implies that considerable care must be given to the interpretation of a thermogravimetric decomposition curve in which the probability of two reactions following one another is recognized. The use of a quasi-static method aids resolution.

The escape of water or other volatile products from a thermally decomposing solid involves a large increase in the entropy of the system (increase in disorder). This factor outweighs the influence of the change in enthalpy (heat content change) of the system, so that an endothermic forward process is possible.

Thus the free energy ΔG is defined by the equation

$$\Delta G = \Delta H - T\Delta S \quad \ldots\ldots\ldots (4)$$

For endothermic reaction under these conditions ΔH is positive and relatively small, $T\Delta S$ is positive and large, and thus $T\Delta S \gg \Delta H$ i.e. ΔG is negative and the reaction will proceed. For an exothermic reaction ΔH is negative and $T\Delta S$ is as before; thus ΔG is still negative and the reaction can occur.

The position of the equilibrium for a reversible change will depend on the decrease in free energy. However even if the reaction takes place in an equilibrium system, the recombination process may be so slow as to make the decomposition virtually complete, i.e. it may be regarded as an irreversible process and the same argument may be applied.

It is important to stress the influence of the recording technique upon a pyrolysis curve (determined on a thermobalance) of a degradation carried out under constant pressure, especially if an attempt is made to interpret the various constants of the processes occurring in solid-solid transformations.

Accurate D.T.A. measurements can be used to provide information regarding the heats of reaction. Although the theoretical treatment is beyond the scope of this article, Smothers and Chiang (1958) have discussed the topic and discussed the applications fully.

REFERENCES

Borchardt and Daniels. (1957). J. Amer. Chem. Soc., **79**, 41.
Freeman and Carroll. (1958). J. Phys. Chem., **62**, 394.
Garner. (1955). 'Chemistry of the Solid State.' Butterworths. London.
Hepburn and Phillips. (1952). J. Chem. Soc., 2569.
Lukaszewski, Redfern and Salmon. (1960). Unpublished work.
Lumme. (1959). Suomen Kemistilehti, **32B**, 198, 237, 241, 253, 261.
Prout and Tompkins. (1944). Trans. Farad. Soc., **40**, 488.
Rollinson. (1956). 'Chemistry of the Co-ordination Compounds.' Ed. J. C. Bailar. Reinhold. New York. Chap. 13.
Shafer and Roy. (1956). J. Amer. Chem. Soc., **78**, 1087.
Smothers and Chiang. (1958). 'Differential Thermal Analysis'. Chem. Pub. Co. New York.
Sullivan and McMurdie. (1952). J. Res. Nat. Bur. Stand., **48**, 159.
Topley. (1932). Proc. Roy. Soc., **136A**, 413.
Ubbelohde. (1957). Quart. Revs., **11**, 246.

BOOK REVIEWS

Paper Electrophoresis. A Review of Methods and Results, by L. P. Ribeiro, E. Mitidieri and O. R. Affonso. London Distributors: D. Van Nostrand Co., Ltd. Amsterdam: Elsevier Publishing Company, 1961. pp. 463 + xii. 72s.

The book is a translation of a revised and enlarged version of a book on paper electrophoresis and related methods, written in Portuguese and published in Rio de Janeiro in 1958. As such it is bound to suffer from certain minor shortcomings due to the lapse of time and difference of style.

Although some references are as recent as 1959, it is quite evident that the comprehensive and thorough treatment of the literature must have stopped not later than 1958. In a fast-moving field of knowledge and applications such as in paper electrophoresis, this may make a book look slightly dated.

On the other hand, due credit must be given to the authors for accomplishing the immense task of preparing a list of 3,226 references, in itself no mean feat. One should have thought that after this the provision of the missing author index would have proved a minor effort.

The concise and clearly-written theoretical part of the book should prove very helpful, even to the reader with little physico-chemical background. The influence of H. J. McDonald's book 'Ionography' (1955) is perhaps too apparent.

More than one-half of the text is devoted to electrophoresis of proteins, particularly serum proteins, in clinical practice. The qualitative and quantitative aspects of their fractionation and determination are lucidly explained, discussed and well illustrated. Particular emphasis is placed upon the discussion of reproducibility of results, accuracy, potential sources of error, and merits or limitations of the various procedures. The authors' views are evidently backed by their wide experience in this field.

Of particular interest and value is the chapter on serum proteins under pathological conditions. Animal serum proteins, haemoglobins, lipoproteins and a host of other proteins are equally expertly described. Electrophoretic techniques in a stabilizing medium other than paper, such as cellulose acetate membranes, starch gel and blocks, etc., are briefly but adequately explained.

A most useful part are the chapters on separations of enzymes, hormones and vitamins, containing highly desirable information on experimental conditions and results of successful applications of electrophoresis to these complex biochemical problems.

It is rather unfortunate that the same degree of competence is not maintained throughout the entire book. The chapters on sugars, amino-acids, peptides, organic acids and inorganic ions are relatively short and create the impression that the authors did not discriminate effectively between essential and trivial information, thus perpetuating in print some of the outdated and already disproved results, whilst omitting some significant more recent contributions. Some obvious mistakes in quotations from papers on carbohydrates could have been avoided with the application of a little more care.

The publishers' name is a sufficient guarantee for excellence of print, make-up and illustrations. The volume can be recommended as a useful edition to any clinical or biological chemist's bookshelf.—D. GROSS.

METHODS FOR THE EVALUATION OF THE ANTI-BACTERIAL ACTIVITY OF SURFACE ACTIVE COMPOUNDS: TECHNICAL ASPECTS OF THE PROBLEM

by J. G. Davis

Dr. J. G. Davis & Partners, 9 Gerrard Street, London, W.1

PART III

5. The Phenol Coefficient Test with QACs

Various explanations have been offered for the anomalies which arise when QACs are tested by the phenol coefficient method, including the following: (i) Unequal distribution of cells due to adsorption (Klarmann and Wright, 1946). (ii) Variations caused by surface tension effects in the volumes of liquids used (Klarmann and Wright, 1946, 1948). (iii) Reactions between constituents of the medium, e.g. phospholipids, and the QAC (Brewer, 1943). (iv) 'Carry over' of QAC in sufficient quantities to exert a bacteriostatic effect. This effect may be reduced if not eliminated by transferring small amounts of the medication liquid to a large excess of growth medium (Klarmann and Wright, 1946; Resuggan and Davis, 1947). (v) Agglutination of the bacteria and adherence of the charged clumps of cells to the glassware (Klarmann and Wright, 1946). The use of plastic, rubber or paraffin wax coated glassware does not seem to give results different from those obtained with ordinary glassware (Lawrence, 1950, p. 56) and the substitution of pipettes for loops only slightly improved reproducibility (Tice and Pressman, 1945). (vi) The occurrence of foaming. Pressman and Rhodes (1946) mixed the solutions by rotation of the tube and, using a pipette transfer plating method, claimed to have obtained very uniform results. (vii) Persistence of a bacteriostatic effect in the growth medium (cf. section 4, c). To avoid any possibility of this, inactivators may be added to the medium (Armbruster and Ridenour, 1947; Quisno *et al.* 1946; Stuart, 1947). (viii) The appearance of false positives, i.e. the occasional occurrence of growth not in conformity with the general pattern of the results. Various theories have been advanced to explain these (cf. Klarmann and Wright, 1946, 1948), but such results must be due to factors such as the survival of the occasionally occurring highly resistant cell, the agglutination of cells to form a cluster which results in the inner cells not being attacked by the germicide, the protection of a few cells by deposition of media constituents, and/or the agglutination of cells, so that the suspension is no longer uniform and the random sampling error of the loop or pipette becomes very large.

Quite apart from criticisms of the phenol coefficient method which apply specifically to surface active compounds, there are other points to be considered. First, if the test organism is by nature resistant to phenol, a high phenol coefficient may be obtained for a substance which is only moderately effective against that organism. Second, the presence of organic matter, use at other temperatures, and other changes in conditions may give markedly different results if the basic phenol coefficient method is thus adapted, e.g. as in the Chick-Martin test. Phenol coefficients for QACs obtained by the F.D.A. method (Ruehle and Brewer, 1931) vary from about 100 to 500 at 20°, and from about 200 to 1,000 at 37° (Lawrence, 1950, p. 64). Third, as the phenol coefficient method expresses a result in terms of pure phenol, preparations which are solutions of germicides and are tested as such therefore appear less effective than if the active substance(s) were tested. While manufacturers doubtless realize this it does not follow that officials and users generally do.

Modifications of the phenol coefficient method to overcome these disadvantages have been proposed by Barber and Harris (1948), Barber *et al.* (1947), Cade (1947a,b), James (1947), Eckfeldt and James (1947), Klimeck and Umbreit (1947), McCulloch (1947a,b), McCulloch *et al.* (1948a,b), Quisno *et al.* (1947a,b).

In assessing the true value of QACs as disinfectants it is essential to give consideration to the rather involved technical arguments advanced by workers in this field. It should also be borne in mind that not all workers and authors are free from commercial bias. The crux of the problem seems to be that ever since 1903 many workers in the disinfectant field have failed to realize that the phenol coefficient method was only intended to form a basis for assessing the power of phenolic disinfectants, or of disinfectants acting like phenol on bacteria. These are less affected by organic matter and have a broader bacterial spectrum than certain other types of disinfectant. Moreover, they are more stable chemically than some.

However, as an example of the usefulness of the test when properly understood, one may quote the practical value of using a disinfectant at a dilution of 20 times the Rideal-Walker coefficient. This is based on the fact that 5 per cent phenol is generally recognized as an efficient disinfectant (Stuart *et al.* 1955).

The logical development of this thesis is that while the basic principle of the Rideal-Walker test, which is the use of a chemical yardstick, should be retained, each group of disinfectants should be considered *de novo* both from the point of view of the chemical yardstick and the end point adopted. The most suitable yardstick would appear to be the simplest of the homologous series for the particular group concerned. The end point should be governed by the purpose for which the disinfectant is designed, and especially whether bactericidal or bacteriostatic properties are the prime

interest. All conditions such as temperature, time, amount and type of organic matter should be adjusted to be as near to the conditions of use as possible.

TABLE VIII

*Possible reference substances for germicide assessment**

Type of germicide	*Reference substance*
Phenols	Phenol
Substances giving 'free chlorine', halogens, iodophors	Sodium hypochlorite (determined as available chlorine)
Quaternary ammonium compounds	Cetyltrimethylammonium chloride
Anionic	Sodium stearate
Ampholytic	Dodecyl-β-alanine
Mercurials	Merthiolate
Alkalis	Sodium hydroxide
Acids	Hydrochloric acid
Alcohols	Ethyl alcohol
Aldehydes	Formaldehyde
Gases and vapours	Ethylene oxide

*The stability of some of these suggested reference substances may be a problem (Hirsch and Muras, 1955).

Possible basic reference substances for various types of germicide are given in Table VIII. On this basis all germicides would be assessed in comparison with the reference substance under defined conditions of testing.

6. Reproducibility

The lack of reproducibility in all tests of QACs, irrespective of the type of test, is well recognized and the question of the number of replicates required is often difficult to answer because of the variety of tests. A simple statistical treatment is possible of the results of tests giving a simple number, e.g. a percentage kill or a limiting dilution, but with tests such as the phenol coefficient, which have wide 'gaps', statistical treatment is more difficult. Lack of reproducibility in colony counts is taken into consideration in the standard formulae for calculating the significance of differences between means, but false positives present a difficult problem. In sorting tests at least three consistent experiments should be made, but making the decision as to when a result is so inconsistent as to be unreliable is difficult. The experienced worker will usually sense an unsatisfactory experiment, but some degree of subjectivity is unavoidable.

It is usually possible to compare two preparations using any one method, and to decide which is superior. Unfortunately another method may reverse this order, and the best procedure then is to base a conclusion on the result given by the method in which the practical conditions of use are most nearly approached.

In all laboratory tests greater accuracy can be expected if the same workers are doing the same tests regularly. The first results by any worker should always be discarded unless they agree with those of subsequent tests. Data on the accuracy of the standard tests have been given by Stuart *et al.* (1958).

We again emphasize that caution should be exercised in the interpretation of any laboratory test of a disinfectant, even if the test is designed to approximate as closely as possible to working conditions. As a guide, we may suggest that if in any laboratory test one disinfectant appears to be at least 10 times as effective as another, then that disinfectant will probably be superior in practice.

7. The Ultimate Objective

All disinfectants and antiseptics are used to prevent the infection of animals or plants, or to prevent the spoilage of food. The ultimate criterion must therefore always be to discover whether the proposed germicide, used as directed, achieves this purpose. No two practical applications of germicides are ever exactly alike, and it is often difficult to standardize all factors under actual working conditions. The value of laboratory tests is threefold; they enable manufacturers and others to make preliminary sorting tests, they permit scientists to compare results from different laboratories, and they enable manufacturers and distributors to quote numerical data in support of their claims.

It will be clear from the evidence which has been adduced on the factors which may affect the efficiency of surface active compounds that the nearer the laboratory test approaches the conditions of use the more valuable will be the test. The quantity assessed should also be the desired objective. For example, if the incidence of infection or the incidence of failures in a food keeping quality test is reduced to nil, it is really of only academic interest if laboratory tests involving inactivation procedures show that pathogenic or food spoilage organisms are surviving in small numbers.

8. Conclusions

Careful study of the available evidence leads to the following conclusions:

(i) No single test is adequate to express either the fundamental or practical value of a disinfectant.

(ii) The QACs vary so much in their properties that it is highly dangerous to generalize about any aspect of their behaviour.

(iii) The nearer the laboratory test approaches the conditions of use, the more reliable the test is likely to be.

(iv) In order to permit estimates of efficiency and to justify claims by manufacturers, one or more standard reference methods should be adopted.

(v) It is unlikely that any one reference method would be acceptable to all manufacturers, testing organizations and users. Each industry or field of interest may require its own reference method, and there should be no objection to this.

(vi) Before accepting a germicide, or a recommended method for using that germicide, use tests should always be made under adverse conditions to ensure that the objective claimed is achieved.

9. Recommendations

It is highly desirable that all purchasers of a disinfectant (using the term in its widest sense) should be given adequate information about its potency. A precise analysis would be useful to chemists, but of little value to others. Moreover, some manufacturers would object to this, and some disinfectants cannot be described in precise chemical terms.

Just as the Labelling of Food Order (1953 Order) requires certain disclosures of composition, and restricts claims for nutritional value, so the Pharmacy and Medicine Act (1941 Act) requires disclosure of the formula for certain types of disinfectant; but with both foods and disinfectants chemical formulae may mean little to the general public, and skilful advertising can get round the legal restrictions.

If it is accepted that it would be impracticable to require disclosure of formula, and that no one test is adequate to assess the value of a germicide, it would be logical to require all manufacturers to state potencies in terms of at least two tests, and at least two types of organism. It should not be difficult for an official and unbiased organization to prepare short lists of such tests and of suitable type cultures. This would not preclude manufacturers from offering information based on the results of other tests, but it would prevent misleading advertising framed on, say, Rideal-Walker tests where such a test result may mean very little, and claims such as a 99·999 per cent kill which, without specifying the technique used, may mean nothing at all.

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11. REFERENCES

Act (1941). Pharmacy and Medicine Act. London: H.M.S.O.
Adam, N. K. and Shute H. L. (1938). Anomalies in the surface tension of paraffin chain salts. Trans. Faraday Soc. **34**, 758.
Allawala, N. A. and Riegelman, S. (1953). The properties of iodine in solutions of surface active agents. J. Amer. pharm. Ass. **42**, 396.
American Dental Association (1932). Mouth washes. J. Amer. dent. Ass. **19**, 1,239.
Armbruster, E. H. and Ridenour, G. M. (1947). A new medium for study of quaternary bactericides. Soap, N.Y., **23**, 119.
Baker, Z., Harrison, R. W. and Miller, B. F. (1941a). The bactericidal action of synthetic detergents. J. exp. Med. **74**, 611.
Baker, Z., Harrison, R. W. and Miller, B. F. (1941b). Inhibition by phospholipids of the action of synthetic detergents on bacteria. J. exp. Med. **74**, 621.
Barber, F. W. and Harris, E. K. (1948). The bacteriological evaluation of several germicides as determined by the oval tube method. Bact. Proc. **1**, 27.
Barber, F. W., Meyers, R. P. and Harris, E. K. (1947). An oval tube method for the determination of the bactericidal effectiveness of various sterilizing agents. J. Bact. **54**, 42.
Bean, H. S. and Berry, H. (1950). The structure and properties of aqueous solutions of soap. J. Pharm. Pharmacol. **2**, 473.
Bean, H. S. and Berry, H. (1951). The bactericidal activity of phenols in aqueous solutions of soap. Part II. The bactericidal activity of benzylchlorophenol in aqueous solutions of potassium laurate. J. Pharm. Pharmacol. **3**, 639.
Berry, H. (1952). The bactericidal action of phenols in aqueous solutions of soap. Proc. Soc. appl. Bact. **15**, 138.
Bettley, F. R. (1960). Some effects of soap on the skin. Brit. med. J. i, 1,675.
Brewer, C. M. (1943). Variations in phenol coefficient determinations of certain disinfectants. Amer. J. publ. Hlth. **33**, 261.
Brewer, J. H. and McLaughlin, C. B. (1951). Use of tetanus spores for testing antiseptics *in vivo*. J. Amer. med. Ass. **146**, 729.
Cade, A. R. (1947a). Increasing efficiency of F.D.A. phenol coefficient test. Soap, N.Y., **23**, 131.
Cade, A. R. (1947b). Revamping the F.D.A. method. Soap, N.Y., **23**, 138.
Chick, H. (1908). An investigation on the laws of disinfection. J. Hyg. Camb., **8**, 92.
Chick, H. (1910). The process of disinfection by chemical agencies and hot water. J. Hyg., Camb., **10**, 237.
Chick, H. (1912). The bactericidal properties of blood serum. J. Hyg., Camb., **12**, 414.
Chick, H. and Martin, C. J. (1908). The principles involved in the standardization of disinfectants and the influence of organic matter upon germicidal value. J. Hyg., Camb., **8**, 654, 698.
Clarke, P. M. and Garvie, E. I. (1955). A factorial investigation of some test conditions in the evaluation of the disinfectant properties of a quaternary ammonium compound using a dried film of *Bact. coli* type 1. J. appl. Bact. **18**, 107.
Clegg, L. F. L. (1955). Laboratory and field evaluation of chemical sterilizers for dairy farms. J. appl. Bact. **18**, 356.
Colebrook, L. and Maxted, W. R. (1933). Antisepsis in midwifery. J. Obstet. Gynaec. **40**, 966.
Cook, A. M. (1954). Comparative studies of methods of evaluating antibacterial substances. J. Pharm. Pharmacol. **6**, 629.
Cousins, C. M. (1951). A preliminary bacteriological screening test for quaternary ammonium compounds and formulations. Proc. Soc. appl. Bact. **14**, 184.
Cousins, C. M. and Clegg, L. F. L. (1956). The effect of water hardness and temperature on water sterilization by mixtures of detergent and quaternary ammonium compounds. J. appl. Bact. **19**, 250.
Cousins, C. M., Hoy, W. A. and Clegg, L. F. L. (1959). The evaluation of the suitability of iodine bearing detergent-sterilizers for farm dairy purposes. Int. Dairy Congr., London, **3**, 1,807.
Cowles, P. B. (1938). The germicidal power of some alcohols for *B. typhosum* and *Staph. aureus* and its relation to surface tension. Yale J. Biol. Med. **11**, 127.
Davies, G. E. (1949). Quaternary ammonium compounds. A new technique for the study of their bactericidal action and the results obtained with Cetavlon (cetyltrimethylammonium bromide). J. Hyg., Camb., **47**, 271.
Davis, J. G. and Resuggan, J. C. L. (1946). The cleaning and sterilization of glasses in licensed premises. Proc. Soc. appl. Bact. (1), 20.
Davis, J. G., Resuggan, J. C. L. and Ive, R. (1949). The disinfection of ice-cream servers. J. R. sanit. Inst. **69**, 439.
Domagk, G. (1935). Eine neue Klasse von Desinfektionsmitteln. Dtsch. med. Wschr. **61**, 829.
Dunn, C. C. (1937). Antiseptic and germicidal properties of a mixture of high molecular alkyl-dimethylbenzylammonium chlorides. Amer. J. Hyg. **26**, 46.
Eckfeldt, G. and James, L. H. (1947). Quaternaries versus phenolics: the germicidal effectiveness of some quaternary and phenolic compounds reported together with time-survival numbers of *E. typhosa* and *S. aureus*. Part 2. Soap, N.Y., **23**, (12), 157.
Einhorn, A. and Goettler, M. (1908). Ueber die Additionsprodukte der Halogenacetamide und deren N-Methylolverbindungen mit Hexamethylentetramin. Liebigs Ann. **361**, 150.
Engler, C. and Diockhoff, E. (1892). The application of soap solutions to disinfectants, with special reference to Lysol. Arch. Pharm., Berl., **230**, 561.
Finch, W. E. (1958). Disinfectants. London: Chapman and Hall.
Garvie, E. I. (1957). The strip test applied to common quaternary ammonium compounds. The effect of different conditions. J. appl. Bact. **20**, 175.
Garvie, E. I. and Clarke, P. M. (1955). The disinfectant properties of quaternary ammonium compounds and sodium hypochlorite: a comparison of results using different test methods. J. appl. Bact. **18**, 90.
Gershenfeld, L. and Milanick, V. E. (1941). Bactericidal and bacteriostatic properties of surface tension depressants. Amer. J. Pharm. **113**, 306.
Gershenfeld, L. and Perlstein, D. (1941). The effects of aerosol OT and hydrogen ion concentration on the bactericidal efficiency of antiseptics. Amer. J. Pharm. **113**, 237.
Glassman, H. N. (1948). Surface active agents and their application in bacteriology. Bact. Rev. **12**, 105.
Goetchius, G. R. (1948). An inexpensive synthetic, non-detergent inactivator for quaternary ammonium germicides. Bact. Proc. **1**, 29.
Goetchius, G. R. and Botwright, W. E. (1950). A new method for the evaluation of quaternary ammonium detergent-sanitizer formulations. J. Milk Fd. Tech. **13**, 63.
Goldberg, A. A., Shapero, M. and Wilder, E. (1950). The penetration of phenylmercuric dinaphthylmethane disulphonate into skin and muscle tissue. J. Pharm. Pharmacol. **2**, 20.
Hartmann, M. and Kägi, H. (1928). Saure Seifen. Z. angew. Chem. **41**, 127.
Heineman, P. G. (1937). Antiseptic properties of alkyldimethylbenzylammonium chloride. J. Amer. pharm. Ass. **26**, 711.
Hirsch, A. and Muras, R. E. (1955). A quantitative method for assessing the effect of disinfectant and cleaning agents on bacteria deposited on surfaces. J. appl. Bact. **18**, 425.
Hornung, H. (1935). Zephirol, ein neues Desinfektionsmittel. Z. Immun-Forsch. **84**, 119.
Hoy, W. A. and Clegg, L. F. L. (1953). Testing farm dairy detergent-sterilizers using soiled ten gallon cans. Proc. Soc. appl. Bact. **16**, 1.
Hucker, G. J., Watkins, S., Metcalf, D. and Stone, J. (1948). Effect of hydrogen-ion concentration and temperature on the activity of quaternary ammonium compounds. Tech. Bull: N.Y. Sta. agric. Exp. Sta. no. 281
Hugo, W. B. (1952). Observations on the manometric estimation of bactericidal activity. Proc. Soc. appl. Bact. **15**, 29.
Jacobs, S. E. and Harris, N. D. (1960). The effect of environmental conditions on the viability and growth of bacteria damaged by phenols. J. appl. Bact. **23**, 294.
Jacobs, W. A. (1916). The bactericidal properties of the quaternary salts of hexamethylenetetramine. I. The problem of the chemotherapy of experimental bacterial infections. J. exp. Med. **23**, 563.

Johns, C. K. (1954). Iodophors as sanitizing agents: Canad. J. Technol. **32**, 71.
Jordan, R. C. and Jacobs, S. E. (1944). Studies in the dynamics of disinfection. I. New data on the reaction between phenol and *Bact. coli* using an improved technique, together with an analysis of the distribution of resistance among the cells of the bacterial populations studied. J. Hyg., Camb., **43**, 275.
Jordan, R. C. and Jacobs, S. E. (1945). Studies in the dynamics of disinfection. III. The reaction between phenol and *Bact. coli*: the effect of temperature and concentration: with a detailed analysis of the reaction velocity. J. Hyg., Camb., **44**, 210.
Klarmann, E. G. and Wright, E. S. (1946). An inquiry into the germicidal performance of quaternary ammonium disinfectants. Soap, N.Y., **22**, 125.
Klarmann, E. G. and Wright, E. S. (1948). Concerning quaternaries: additional data support 'semi-micro' technic in testing quaternary germicides. Soap, N.Y., **24**, 155.
Klimek, J. W. and Umbreit, L. E. (1947). On the germicidal behaviour of a quaternary ammonium compound. Soap, N.Y., **23**, 125.
Klein, M. and Kardon, Z. G. (1947). The 'reversal' neutralization, and selectivity of germicidal cationic detergents. J. Bact. **54**, 245.
Kliewe, H. and Maier, E. (1936) Vergleichende Untersuchungen über die gebrauchlichen Desinfektionsmittel. Munch. med. Wschr. **83**, 299.
Lawrence, C. A. (1947). Inactivation of the germicidal action of quaternary ammonium compounds. J. Bact. **53**, 375.
Lawrence, C. A. (1948). Inactivation of the germicidal action of quaternary ammonium compounds. J. Amer. pharm. Ass. **37**, 57.
Lawrence, C. A. (1950). Surface Active Quaternary Ammonium Germicides. New York: Academic Press, Inc.
Lawrence, C. A. and Erlandson, A. L. (1950). A new activating medium for hexachlorophene (G-11). J. Amer. pharm. Ass. **42**, 352.
Lister, J. (1867). On a new method of treating compound fracture, abscess, etc. with observations on the condition of suppuration. Lancet **i**, 326, 352, 387, 507; **ii**, 95.
McBain, J. W. (1913). Mobility of highly charged micelles. Trans. Faraday Soc. **9**, 99.
McCulloch, E. C. (1945). Disinfection and Sterilization. London: Henry Kimpton.
McCulloch, E. C. (1947a). The real and the apparent bactericidal efficiencies of the quaternary ammonium compounds. J. Bact. **53**, 370.
McCulloch, E. C. (1947b). False disinfection velocity curves produced by quaternary ammonium compounds. Science **105**, 480.
McCulloch, E. C., Hauge, S. and Migaki, H. (1948a). The role of disinfectants in veterinary medicine. II. A critical evaluation of the quaternary ammonium compounds. J. Amer. vet. med. Ass. **112**, 283.
McCulloch, E. C., Hauge, S. and Migaki, H. (1948b). The quaternary ammonium compounds in sanitization. Amer. J. publ. Hlth. **38**, 493.
McCutcheon, J. W. (1955). Synthetic detergents and emulsifiers—up to date. Soap, N.Y., **31**, (7), 50, (8), 48, (9), 52, (10), 48.
Mallman, W. L. and Hanes, M. (1945). The use-dilution method of testing disinfectants. J. Bact. **49**, 526.
Marshall, M. S. and Krenoff, A. K. (1937). Bacteriostases. J. infect. Dis. **61**, 42.
Maurice, P. (1952). A substitute for measurement of viability in the evaluation of disinfectants. Proc. Soc. appl. Bact. **15**, 144.
Meewes, K. H. (1941). Studien über probleme bei der prüfung von Desinfektionsmitteln. Z. Hyg. InfektKr. **123**, 239.
Moore, C. D. (1960). Ampholytic surface active agents. J. Soc. cosmetic Chem. **11**, 1.
Moore, C. D. and Hardwick, R. B. (1958). Germicides based on surface active agents. Mfg. Chem. **29**, 194.
Mueller, W. S., Bennett, E. and Fuller, J. E. (1946). Bactericidal properties of some surface-active agents. J. Dairy Sci. **29**, 751.
Nagel, A. (1940). Ueber die desinfizierende Wirkung des Blattezephirols (I.G.). Münch. med. Wschr. **36**, 970.
Neave, F. K. and Hoy, W. A. (1947). The disinfection of contaminated metal surfaces with hypochlorite solutions. J. Dairy Res. **15**, 24.
Order (1953). Labelling of Food Order. London: H.M.S.O.
Ortenzio, L. F. (1958). Report on fungicides and subculture media (Chambers modification of the Weber and Black test). J. Ass. off. agric. Chem., Wash., **41**, 541.
Ortenzio, L. F., Caswell, R. L., Friedl, J. L. and Stuart, L. S. (1954). Detergent residues and their effect on disinfecting processes. Proc. Chem. Spec. Manuf. Ass., Cincinnati, Ohio, p. 82.
Phelps, E. B. (1911). The application of certain laws of physical chemistry in the standardization of disinfectants. J. infect. Dis. **8**, 27.
Pressman, R. and Rhodes, J. C. (1946). Sources of error in germicidal activity tests with quaternary ammonium compounds. Soap, N.Y., **22**, 137.
Price, P. B. (1950). The meaning of bacteriostatis, bactericidal effect,and rate of disinfection. Ann. N.Y. Acad. Sci. **53**, 76.
Quisno, R. and Foter, M. J. (1946). Cetylpyridinium chloride. J. Bact. **52**, 111.
Quisno, R., Gibby, I. W. and Foter, M. J. (1946). A neutralizing medium for evaluating the germicidal potency of quaternary ammonium compounds. Amer. J. Pharm. **118**, 320.
Quisno, R., Foter, M. J. and Rubenkoenig, H. L. (1947a). Difficulties involved in the evaluation of quaternary ammonium compounds. J. Bact. **54**, 43.
Jacobs, W. A., Heidelberger, M. and Amoss, H. L. (1916a). The bactericidal properties of the of the quaternary salts of hexamethylenetetramine. II. The relation between constitution and bactericidal action in the substituted benzylhexamethylenetetraminium salts. J. exp. Med. **23**, 569.
Jacobs, W. A., Heidelberger, M. and Bull, C. G. (1916b). The bactericidal properties of the quaternary salts of hexamethylenetetramine. III. The relation between constitution and bactericidal action in the quaternary salts obtained from halogenacetyl compounds. J. exp. Med. **23**, 577.
James, L. H. (1947). Quaternaries versus phenolics: the germicidal effectiveness of some quaternary and phenolic compounds reported with time-survival numbers of *E. typhosa* and *S. aureus*. Soap, N.Y., **23**, (11), 125.
Jensen, V. and Jensen, E. (1933). Determination of the phenol coefficient of disinfectants by the coverslip method. J. Hyg., Camb., **33**, 485.
Johns, C. K. (1947). A method for assessing the sanitizing efficiency of quaternary ammonium and hypochlorite products. Amer. J. publ. Hlth. **37**, 1,322.
Johns, C. K. (1948). Germicides for the food industry. Canad. Fd. Ind. **19**, 24.
Quisno, R., Foter, M. J. and Rubenkoenig, H. L. (1947b). Quaternary ammonium germicides: a discussion of methods for their evaluation. Soap, N.Y., **23**, 145.
Ralston, A. W. (1946). Surface-active agents: the structure and properties of solutions of colloidal electrolytes. Ann. Sci. **46**, 351.
Ralston, A. W., Hoerr, C. W. and Hoffman, E. J. (1942). Studies on high molecular weight aliphatic amines and their salts. IV. Electrical conductivities of aqueous solutions of the hydrochlorides and acetates of dodecyl and octadecylamines. J. Amer. Chem. Soc. **64**, 97.
Rahn, O. (1946). Protection of dry bacteria against cationic detergents. Proc. Soc. exp. Biol., N.Y., **62**, 2.
Rahn, O. (1947). Advisability of some proposed changes for the evaluation of disinfectants. J. Amer. pharm. Ass. **36**, 134.
Reddish, G. F. (ed.) (1957). Antiseptics, Disinfectants, Fungicides and Physical Sterilization. London: Henry Kimpton.
Reid, H. (1959). Skin disinfection. Brit. med. J. ii 697.
Reid, H., Black, T. and Dean, D. (1958). The evaluation of skin antiseptics. Practitioner **180**, 707.
Report (1947). Interfering action of tap waters on germicidal properties of quaternary ammonium compounds. Rep. Conf. sanit. Engng. Div. publ. Hlth. Serv., Wash., May 7-8.
Resuggan, J. C. L. (1951). Quaternary Ammonium Compounds. London: United Trade Press Ltd.
Resuggan, J. C. L. and Davis, J. G. (1947). A new approach to the bottle-washing problem. Dairy Ind. **12**, 443.
Reychler, A. (1913). Sur quelques substances contenant le radical cétyle: L'iodure de triethylcetyl-ammonium. Bull. Soc. chim. Belg. **27**, 217.
Rideal, S. and Walker, J. T. A. (1903). The standardization of disinfectants. J. R. sanit. Inst. **24**, 424.
Ruehle, G. L. A. and Brewer, C. M. (1931). Methods of testing antiseptics and disinfectants. Circ. U.S. Dep. Agric. no. 198.
Salton, M. R. J. (1950). The bactericidal properties of certain cationic detergents. Aust. J. sci. Res. **B 3**, 45.
Schmitz, A. and Harris, W. S. (1958). Germicidal ampholytic surface-active agents. Mfg. Chem. **29**, 51.
Schwartz, A. M., Perry, J. W. and Berch, J. (1958). Surface-Active Agents and Detergents, Vol. 2. New York: Interscience Publishers Inc.
Senn, N. (1905). Iodine in surgery, with special reference to its use as an antiseptic. Surg. Gynec. Obstet. **I**, 1.
Society for Dairy Technology (1959). In-Place Cleaning of Dairy Equipment. London: Soc. Dairy Tech.
Soike, K. F., Miller, D. D. and Elliker, P. R. (1952). Effect of pH of solution on germicidal activity of quaternary ammonium compounds. J. Dairy Sci. **35**, 764.
Specification (1934). Technique for determining the Rideal-Walker coefficient of disinfectants. British Standard Specification no. 541. London: British Standards Institution.
Steingold, L., Wood, J. H. F. and Finch, W. E. (1954). Observations on the disinfection of hospital blankets with quaternary ammonium compounds during laundering. J. appl. Bact. **17**, 159.
Stuart, L. S. (1947). N.A.I.D.M.-U.S.D.A. report on quaternary ammonium testing. Soap, N.Y., **23**, 135.
Stuart, L. S., Ortenzio, L. F. and Friedl, J. L. (1955). The phenol coefficient number as an index to the practical use-dilution for disinfection. J. Ass. off. agric. Chem., Wash., **38**, 465.
Stuart, L. S., Ortenzio, L. F. and Friedl, J. L. (1958). Variations in phenol coefficient testing. Soap, N.Y., **34** (10), 79.
Suter, C. M. (1941). Relationships between the structures and bactericidal properties of phenols. Chem. Rev. **28**, 269.
Sykes, G. (1958). Disinfection and Sterilization. London: E. and F. N. Spon.
Tice, L. F. and Pressman, R. (1945). Antiseptics of the quaternary ammonium type in the presence of positive and negative gelatin. J. Amer. pharm. Ass. **34**, 201.
Valko, E. I. (1946). Surface-active agents in biology and medicine. Ann. N.Y. Acad. Sci. **46**, 451.
Valko, E. I. and Dubois, A. S. (1944). The anti-bacterial action of surface active cations. J. Bact. **47**, 15.
Valko, E. I. and Dubois, A. S. (1945). Correlation between anti-bacterial power and chemical structure of higher alkyl ammonium ions. J. Bact. **50**, 481.
Vincent, J. G. and Vincent, H. W. (1944). Filter paper disc modification of the Oxford cup penicillin determination. Proc. Soc. exp. Biol., N.Y., **55**, 162.
Walker, J. E. (1924). The germicidal properties of chemically pure soaps. J. infect. Dis. **35**, 557.
Walker, J. E. (1925). The germicidal properties of soap. J. infect. Dis. **37**, 181.
Walker, J. E. (1926). The germicidal properties of soap. J. infect. Dis. **38**, 127.
Welch, H. and Brewer, C. M. (1942). Relative toxicity of certain antiseptics containing soap and alcohol: with special references to mouth washes. Amer. J. publ. Hlth. **32**, 261.
Whitt, E. W. (1958). A surface film method of evaluating quaternary ammonium compounds. J. appl. Bact. **21**, 272.
Withell, E. R. (1942). Evaluation of bactericides. J. Hyg., Camb., **42**, 339.
Wolf, J. and Brown, J. (1946). A note on the germicidal properties of some mouth washes. Proc. Soc. appl. Bact. (2), 99.

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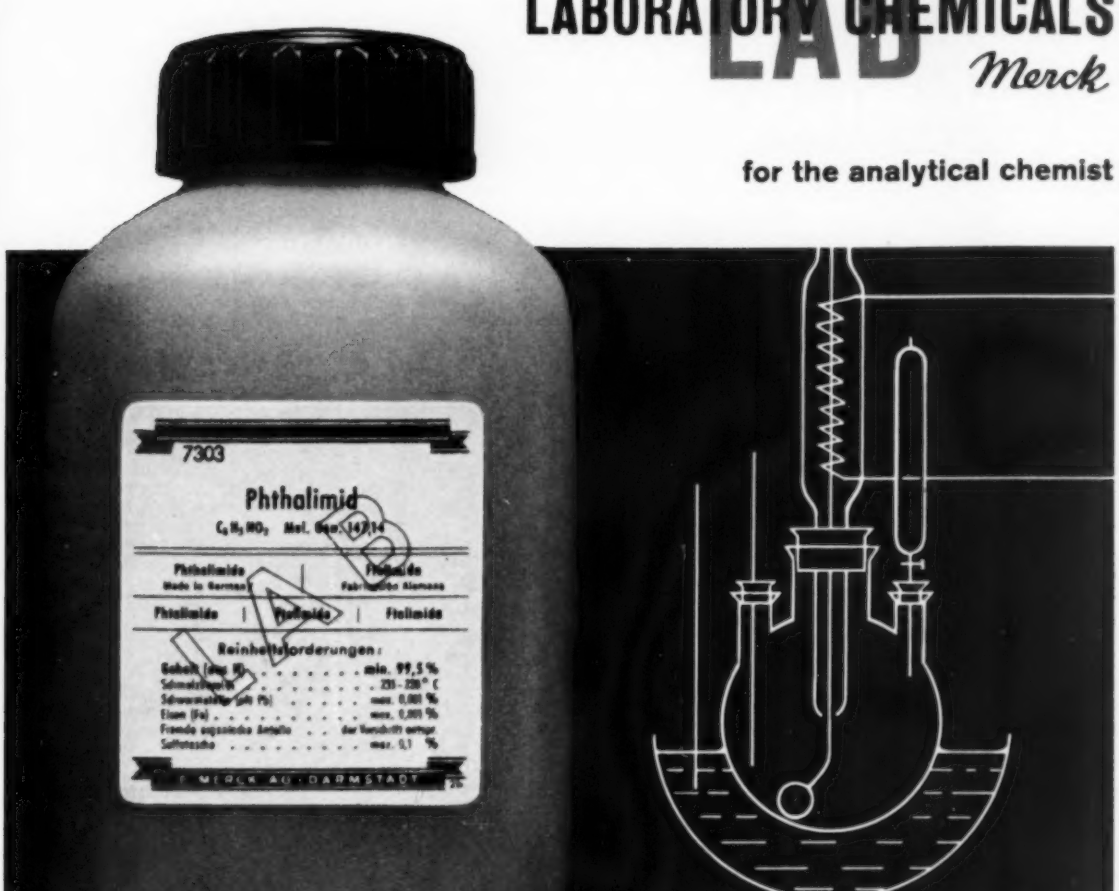

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LABORATORY EQUIPMENT & APPLIANCES

A New Laboratory Grinding Mill

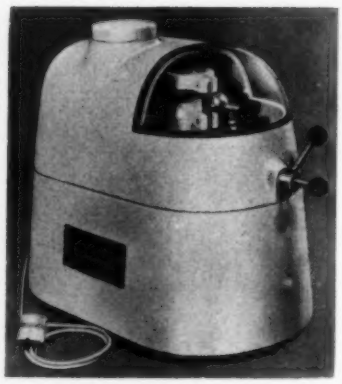
A universal laboratory mill for the dry or wet grinding of batch samples of solid substances to the degree required for analysis purposes has now been developed.

The mortar has a volume of 625 cu. cm. and, when the mill is closed, accommodates about 100 cu. cm. material with an initial maximum particle size of about 5 mm. and if the lid is not completely closed, the mill can pre-grind from larger size. The grinding components proper (mortar and pestle) are made, according to choice, of laboratory porcelain, steel (either hardened or stainless acid-resistant), 'AF-sintered sapphire' (in two grades), agate or tungsten-carbide (WC+Co). For analytical applications, where abrasion must be kept to a minimum, and for the long-term grinding of samples of a highly abrasive material, agate is recommended because of its high wear resistance. Agate (SiO_2) is a relatively good heat conductor, so components of this material are also recommended for grinding heat-sensitive substances. For cases where the abrasive strength even of agate is insufficient, e.g. in trace analysis, tungsten-carbide is the most abrasion- and wear-resistant material known. It contains less than 0·1 per cent iron, but is costly.

The grinding components (mortar and pestle) can obviously be cleaned easily because smooth surfaces only are involved. They can moreover be replaced in five seconds which is an advantage particularly when various sets of grinding components are used alternately.

Grinding pressure can be adjusted and later re-set to the same value by means of a sliding weight, hard material being ground under high, and 'sticky' material under low grinding pressure (Registered Design). A special double scraper has been designed for grinding/mixing/homogenizing/dispersing very small samples (maximum 10 cu. cm.), of materials that are very difficult to grind ('sticky' substances), e.g. pigments, resins, graphite, etc.

The mortar is enclosed up to the upper rim and thereby protected and kept in position. The grinding process can be observed through a large Plexiglass window in the lid. Below this, a transparent hood immediately above the mortar prevents the ejection of material during grinding (German Patent). In addition, the hood makes a dust-proof seal with the frame so that even corrosive and dusty samples can be ground without loss. The motor and grinding cavity are enclosed so that the machine operates with a minimum of noise.



The Pulverisette 2 grinding mill.

The mill is fitted with a time switch to provide fully automatic operation once the material has been fed in and the grinding pressure set.

As regards economy of operation, it should be emphasized that the laboratory mill described has been proved in practice and, by virtue of its design, represents genuine rationalization of laboratory grinding so that the machine will pay for itself very quickly. Laborious hand grinding and inaccurate results are eliminated; the results obtained are precise and may be repeated as desired. (Manufacturers: Alfred Fritsch, Idar-Oberstein 1, Hauptstrasse 542, W. Germany; Sole Agents: V. A. Howe & Co., Ltd., 46 Pembridge Road, London, W.11.)

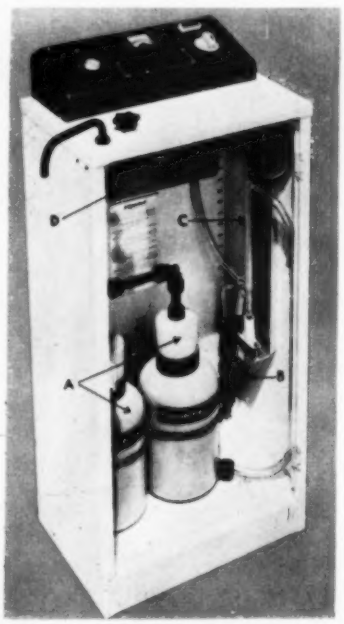
Mixed Bed Deionizer with U.V. Sterilizer

The new Elgastat B.112/UV mixed bed deionizer provides purified water B.P., ultra pure water, and sterile deionized water for pharmacy, biochemistry, physiology, cytology, biology, agricultural and horticultural research and chemistry.

To prevent any possible organisms entering the system, the tap water first passes through an ultra violet tube. From there it is guided through two mixed bed ion exchange cartridges. The deionized effluent passes through a second channel in the U.V. tube to the draw off point.

A conductivity meter indicates effluent quality throughout usage. Effluent purity is consistent at a minimum of 10 megohm-cm. For sterile effluent, the U.V. lamp is switched on and a panel signal confirms its operation.

Flow rate is up to 250 litres per hour. No regeneration *in situ* is essential, the Elgastat cartridge service applies to this deionizer. Yield between cartridge exchanges depends upon the dissolved solids in the raw water; in hard water areas some 400 litres may be drawn, in soft water areas up to 4,000 litres of sterile deionized effluent are available between cartridge exchanges. To maintain sterility, the draw off pipe is removable for sterilizing. The unit is fully mobile on castors and measures 42 in. × 18 in. × 13 in. Price is £150. (Manufacturers: Elga Products Ltd., Lane End, Bucks.)



In the new Elgastat B.112/UV mixed bed deionizer illustrated on the right the main water enters at a point at the back of the unit and is drawn from the outlet next to the valve on top of the casing. A—two mixed bed ion exchange cartridges in series. B—conductivity cell. C—two channel U.V. sterilizer. D—transformer.

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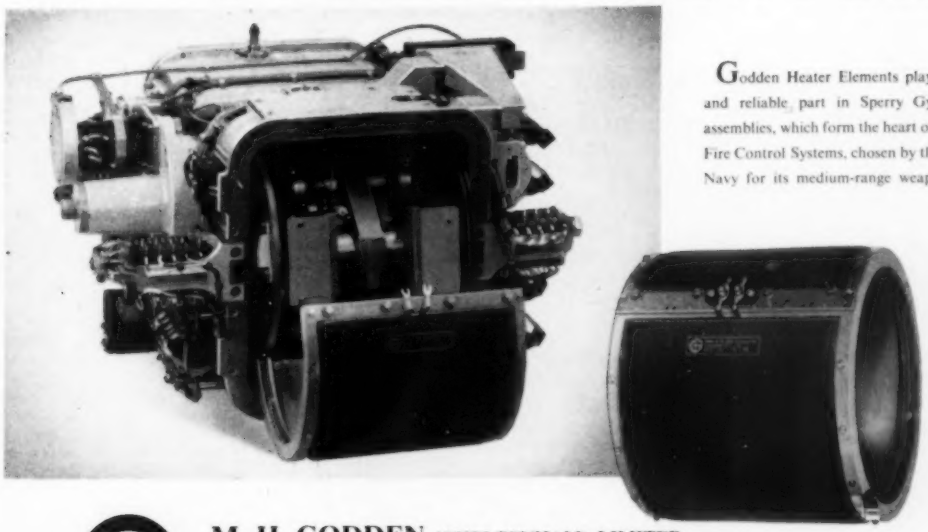

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NEWS

General

New School Science Block

Girls are taking a growing interest in chemistry and scientific subjects in schools, but need the added encouragement of pleasant, light and airy laboratories which many schools have lacked in the past. This is the view of Mr. R. G. Jones, B.Sc., master in charge of chemistry at St. Christopher School, Letchworth.

Looking round the new science block at the school, which has been in use for only a few weeks, he forecast that this purpose-built department would attract more and more girls.

An acoustic-tiled ceiling, ventilating fans, and wide picture windows overlooking the school playing field make the main laboratory a pleasant workroom. Careful design of the benches, with a central trough, individual storage drawers and cupboards, Bunsen burner points and service outlets, ensure that all the necessary facilities are available for a class of 20 students.

Commonly used chemicals are kept in two bays of shelving at one end of the laboratory, separated by a wide screen for the projection of demonstration films and slides. To cope with the many different sizes and shapes of bottles required, the Spur adjustable shelving system has been incorporated, not only in the main laboratory, but in a storage room to one side and in another adjoining room where reference books are stored.

Grey Spur wall-mounted uprights and brackets are used throughout, with 300 ft. of matching Spur metal shelves, on which are accommodated stocks of chemicals ample for instruction in chemistry subjects up to University Entrance standards.

Architect for the new science block was William E. Barnes, F.R.I.B.A. The shelving used in the laboratory and adjoining rooms was manufactured by Savage & Parsons Ltd. and supplied through the Standard Range and Foundry Co. Ltd.

A pupil at St. Christopher School, Letchworth, conducts an experiment in the new science block.

Personal Notes

Shippam Appointments

With the continued expansion of the company two new Directors have been elected to the Board of C. Shippam Ltd.

Dr. F. H. Banfield becomes Technical Director and Mr. Peter H. Fleck, Marketing Director.

Dr. Banfield, who has been at the British Food Manufacturing Industries' Research Association for 35 years, and Director for the past 10 years, is of course a leading figure not only in the food industry in this country but also in food manufacturing circles all over the world. Mr. Fleck has been the Company's Marketing Manager for the past four years.

CATALOGUES, BROCHURES & LEAFLETS

Received from Manufacturers

Laboratory Furnishings.—Camlab (Glass) Ltd., of Milton Road, Cambridge, have issued the second edition of their catalogue, a substantial part of which has been devoted to both new and capital equipment. As laboratory furnishers, the company hold extensive stocks of every-day requirements and the company will always be pleased to forward up-to-date price lists from the main producers.

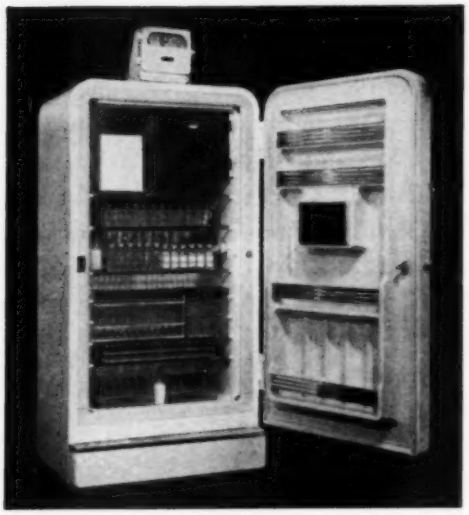
Laboratory Chemicals.—The British Drug Houses Ltd. of Poole, Dorset, have just issued their 1961 catalogue (354 pages) of laboratory chemicals.

Lead for Atomics.—In a new brochure —'Lead for Radiation Protection'— Associated Lead make available in concise form up-to-date information about lead in the field of atomics. This brochure gives much technical information on the mechanical properties of lead, the forms of lead shielding bricks available and some of the alternative methods of providing reactor shielding. A section is also devoted to a new material called Densithene which is now being produced by Associated Lead and which is a composite lead/polythene product of particular importance in dealing with neutron and gamma-ray shielding. A special section deals with the use of lead in x-ray work. This booklet is available from Associated Lead Manufacturers Ltd., Clements House, 14 Gresham Street, E.C.2.

Engineering Laboratory Equipment.—A combined density and moisture surface meter of special interest to engineering laboratories, is one of many new and varied instruments illustrated in a catalogue just issued by Engineering Laboratory Equipment Ltd., 285 Ealing Road, Alperton, Wembley, Middlesex.

The catalogue (Publication No. P. 2122) comprises eight sections covering such varied subjects as soils, concrete and powders. There are sections also on mobile laboratories for use on sites by building and civil engineering contractors and public authorities.

Carl Zeiss Works Magazine.—The Carl Zeiss Werkzeitschrift contains much to interest the scientist. Articles in the current issue include 'Electron Beam Tools of Modern Technology' by K. H. Steigerwald, 'Use of Low-magnification Luminars for Survey Photographs' by Dr. J. C. van de Kamer, 'Use of Optical Measuring Instruments in Breweries', and an article by Prof. M. Diem 'Concerning the Measuring Technique of Cloud Structure and Precipitation'. The magazine is published in English by the Carl Zeiss Foundation, and is available free of charge from Degenhardt & Co. Ltd., U.K. agents for Carl Zeiss, 6 Cavendish Square, W.1.



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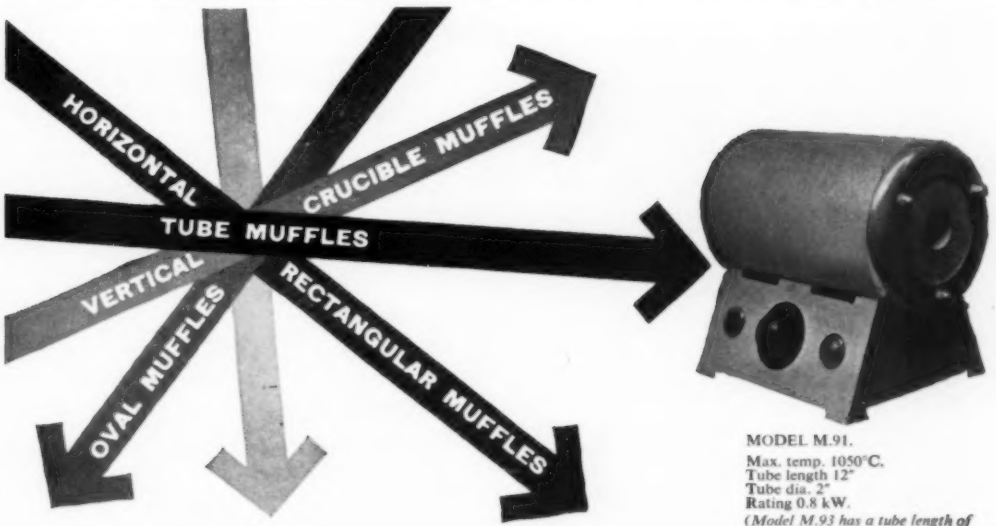

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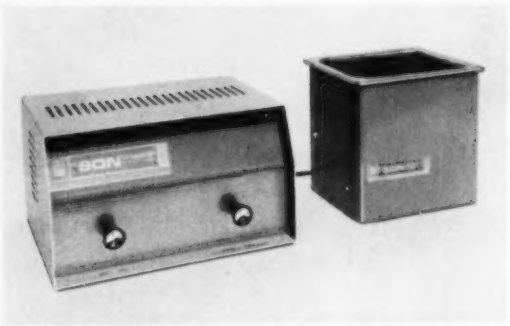

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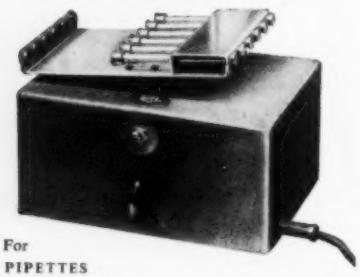

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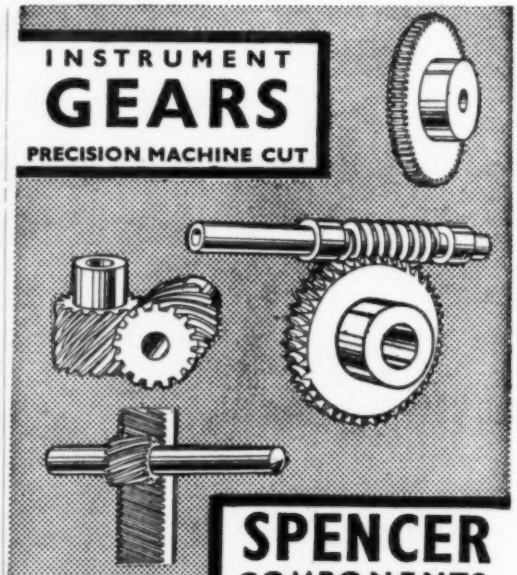

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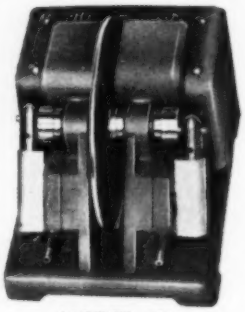

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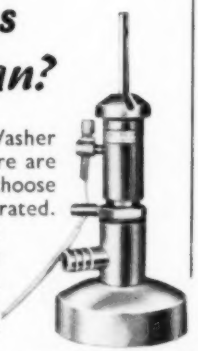

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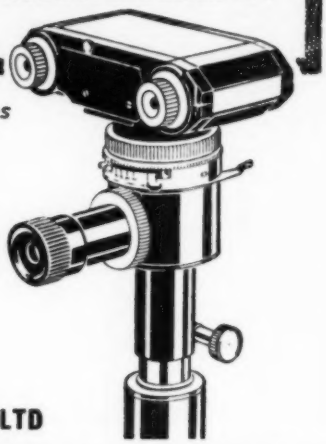

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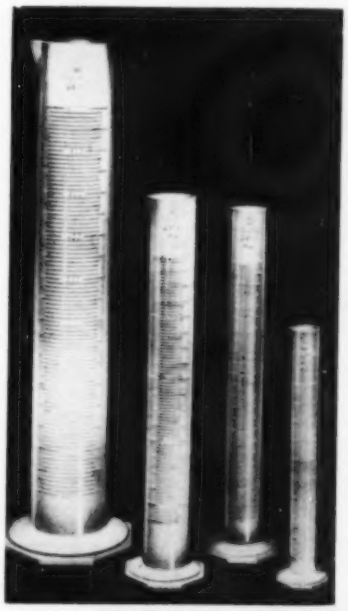

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Applicants should possess the Associateship or Fellowship qualifications of the I.M.L.T. (Diploma in Pathological Technique essential).

Salary scales: With Fellowship £1,210—£1,500 per annum; With Associateship £1,075—£1,300 per annum.

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**University of Otago,
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Department of Chemical Pathology.**
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Salary scales: With Fellowship £1,210—£1,500 per annum; With Associateship £1,075—£1,300 per annum.

Further particulars may be obtained from the Secretary, Association of Universities, of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close on October 31, 1961.

**University of Otago,
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Haematology and Blood Serology Departments.**
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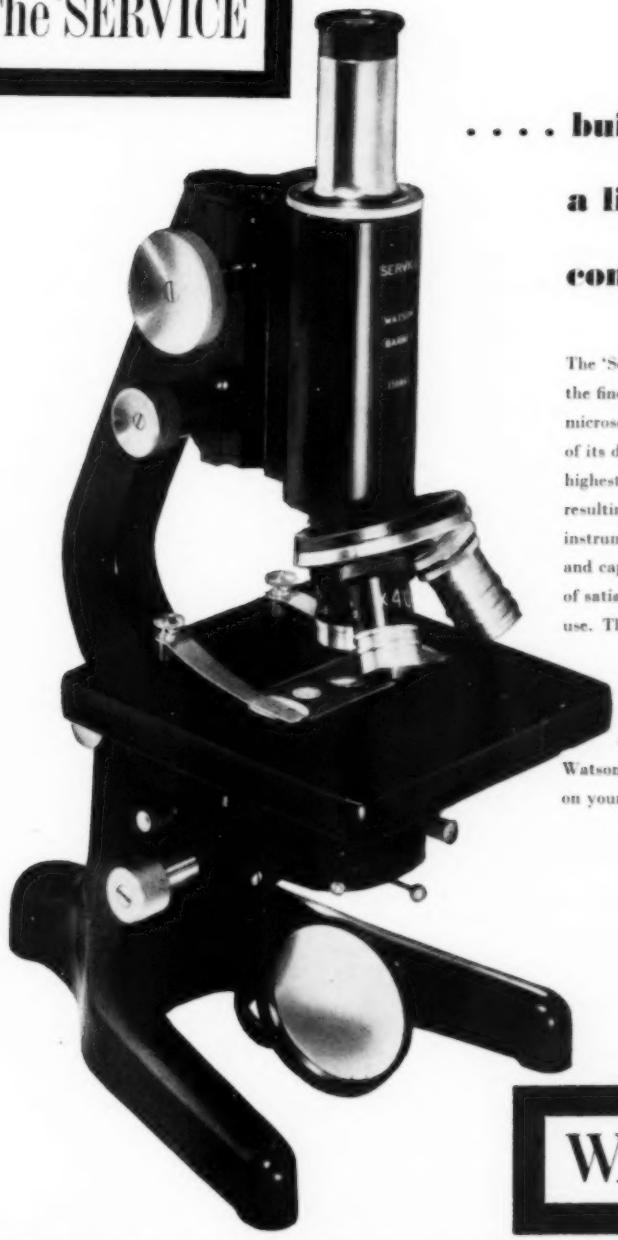

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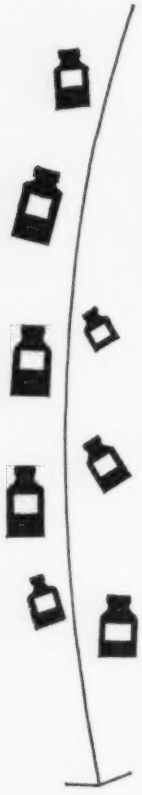

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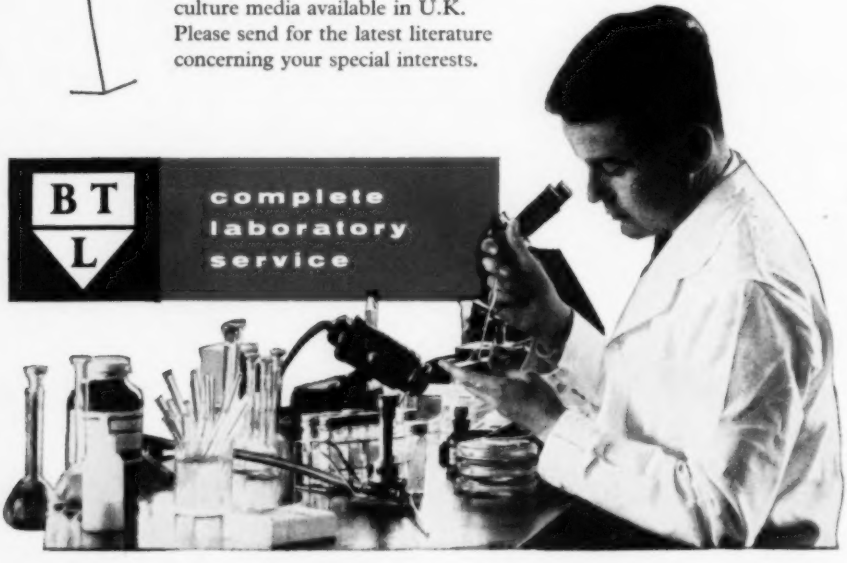

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